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Genetic Properties of Chromosomally Integrated 2μ Plasmid DNA In Yeast

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Summary

We obtained strains of yeast with large segments of 2μ plasmid DNA integrated at several chromosomal locations by selecting genetically for recombination between a chromosomal sequence carried on a 2μ-circle-containing hybrid plasmid and a homologous sequence on the chromosome. In all diploids examined, the presence of 2μ circle sequences causes a marked instability of the chromosome into which the 2μ DNA is inserted. Although in some cases the loss of genetic markers is due to physical loss of the entire chromosome, in most cases the loss of markers appears to be due to a mitotic homozygotization of markers: the allelic information from the homologous chromosome replaces the information distal to the integrated 2μ DNA. The instability caused by integrated 2μ DNA sequences requires the activity of the specialized site-specific recombination system encoded by the 2μ plasmid. We propose that the presence of integrated 2μ DNA allows efficient integration of additional copies of the intact 2μ plasmid by the action of the plasmid-coded special recombination system. Unequal sister-strand exchanges within the inverted repetition would result in the formation of dicentric chromosomes whose breakage during mitosis might begin a cycle analogous to the breakage-fusion-bridge cycle described many years ago in maize.

Introduction

The 2μ circular DNA plasmid of yeast (Saccharomyces cerevisiae) has been studied extensively because of the information it can provide about eucaryotic DNA replication and site-specific recombination and because of its usefulness in the construction of cloning vectors (Broach, 1981). As its name indicates, the 2μ plasmid is a circular duplex molecule with a molecular length of about 2 μm (approximately 6 kb). The cellular function, if any, of the 2μ DNA is not known. The plasmid is present in 50–100 copies per cell in most strains of Saccharomyces cerevisiae (Bak et al., 1972; Clark-Walker and Miklos, 1974). The nucleotide sequence of the entire 2μ circle DNA (6318 bp) has recently been determined (Hartley and Donelson, 1980). It is inherited in a non-Mendelian fashion (Livingston, 1977); for this reason it has often been regarded as a "cytoplasmic" element. However, much recent evidence indicates that the plasmid is actually located in the nucleus (Livingston and Hahne, 1979; Nelson and Fangman, 1979; Kielland-Brandt et al., 1980; Taketo et al., 1980). We provide further evidence on this point below by showing that the 2μ DNA can recombine readily with nuclear DNA.

The most remarkable structural feature of the 2μ plasmid DNA molecule is the presence of an inverted repetition (599 bp long) that separates the unique regions into two domains (Beggs et al., 1976; Guerineau et al., 1976; Hollenberg et al., 1976; Gubbins et al., 1977; Livingston and Klein, 1977; Hartley and Donelson, 1980). Intramolecular recombination within the inverted repetition occurs frequently, resulting in a mixed population of two forms of the 2μ DNA molecule, designated forms A and B (Beggs, 1978). A site within the inverted repetition that is necessary for the high-frequency interconversion of the two forms has been identified (Broach et al., 1982). In addition, a site required for autonomous replication of the plasmid (presumably the origin of replication) has been localized to a region overlapping the end of one of the repetitions and continuing into the adjacent unique region (Broach and Hicks, 1980).

Much of the 2μ circle genome is transcribed into RNAs that can be detected as several distinct polyadenylated species in cell extracts (Broach et al., 1979). There are three large open translational reading frames that could code for proteins of molecular weights greater than 30,000 (Hartley and Donelson, 1980). Three structural genes have thus far been assigned to regions containing open reading frames in the 2μ plasmid genome: the *REP* genes, which code for proteins that are necessary to maintain a high copy number of 2μ DNA molecules, and the *FLP* gene, which encodes a diffusible function required for the high-frequency site-specific recombination within the inverted repetition (Broach and Hicks, 1980; Broach, 1981).

We studied homologous recombination of sequences attached to 2μ plasmids with chromosomal sequences in yeast. A detailed account of these studies will be presented elsewhere (S. C. Falco, M. Rose and D. Botstein, in preparation). Among the recombinants we observed were cases in which plasmids (including 2μ DNA) had integrated into a yeast chromosome. Chromosomal insertion of 2μ circle DNA has interesting genetic consequences for the chromosome into which it integrates. A description of these consequences, a characterization of the essential elements and a discussion of the possible mechanisms involved are the subjects of this report. Kielland-Brandt et al. (1980) have recently reported evidence

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for chromosomal integration of 2μ circle DNA by homologous recombination also.

Results

Most of the experiments described in this paper involve two very similar hybrid plasmids containing 2μ DNA, whose structures are shown in Figure 1. Each plasmid is derived from the common bacterial cloning vector pBR322 (Bolivar et al., 1977); each retains a functional gene encoding ampicillin resistance (Ap^r), but both are lacking the tetracycline resistance determinant owing to the insertion of yeast DNA. Each plasmid contains three fragments of yeast DNA: first, a 2.3 kb fragment carrying the yeast *LEU2*$^+$ gene (Botstein et al., 1979), which is used to select for the presence of the plasmid in yeast; second, a 5.5 kb fragment (Rose et al., 1981; M. Rose, unpublished results) containing a mutant allele (*ura3-3*) of the yeast *URA3* gene; and third, a portion of the 2μ circle DNA.

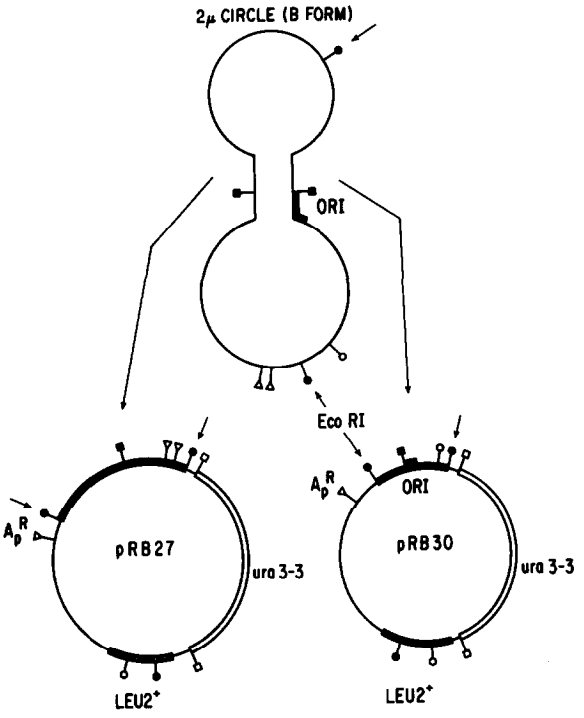

Figure 1. Diagram of the Yeast 2μ Circle Plasmid and Plasmids pRB27 and pRB30

Plasmids pRB27 and pRB30 are derived from pBR322 and contain a 5.5 kb Bam HI restriction fragment that includes the *ura3-3* amber allele and a 2.3 kb Sal I to Xho I restriction fragment that includes the *LEU2* gene. pRB27 contains the large (4.1 kb) Eco RI fragment from the yeast 2μ plasmid B form, which lacks the 2μ circle origin of DNA replication. pRB30 contains the small (2.2 kb) Eco RI fragment from the 2μ circle B form, which incudes the 2μ plasmid origin of DNA replication. Both Eco RI fragments contain a copy of the 599 bp inverted repetition of 2μ circle, which are shown aligned. pRB27 and pRB30 were constructed by digesting the previously described plasmids YEp20 and YEp24, respectively (Botstein et al., 1979), with Bam H1 and ligating them to the 5.5 kb yeast DNA Bam HI restriction fragment containing the yeast *ura3-3* allele. Isolation of the latter fragment will be described elsewhere (M. Rose and D. Botstein, in preparation). Restriction sites are as follows. (●) Eco RI. (○) Hap I. (■) Xba I. (□) Bam HI. (▽) Pvu II.

The 2μ plasmid is cleaved into two unequal halves by the restriction endonuclease Eco RI. As shown in Figure 1, plasmid pRB27 contains the larger (approximately 4.1 kb) Eco RI fragment of the B form (Beggs, 1978) of 2μ DNA, and pRB30 contains the smaller (approximately 2.2 kb) fragment. Each of these Eco RI fragments contains a single complete copy of the inverted repetition (Beggs, 1978; Hartley and Donelson, 1980). The small fragment in pRB30 also contains the putative origin of replication (referred to as *ORI*$^+$; Broach and Hicks, 1980). Although the 4.1 Eco RI fragment in pRB27 lacks the 2μ circle origin of replication (that is, pRB27 is *ORI*$^-$), the 2μ DNA fragment nevertheless confers upon pRB27 a high frequency of transformation in yeast and allows autonomous replication of pRB27 in yeast through efficient recombination (promoted by the *FLP* gene) with resident 2μ plasmids (Broach and Hicks, 1980).

Each of the plasmids was introduced into the haploid yeast strain DBY931 (a haploid strain containing 2μ DNA [*cir*$^+$] and carrying a double mutation in *leu2* and the *ura3-50* mutation as well as other markers) by transformation, selecting for leucine prototrophy (Hinnen et al., 1978). Transformants were purified and subcloned in such a way as to maintain selection for the unstable plasmid by constantly requiring the Leu$^+$ phenotype. All transformants were Ura$^-$ in phenotype, since both the plasmid-borne and chromosomal copies of the *URA3* gene are mutant. Independent Ura$^+$ recombinants were selected from individual subclones. Three classes of recombinants were distinguished on the basis of the stability of the Ura$^+$ and Leu$^+$ phenotypes; a detailed account of the features of such plasmid–chromosome recombination will be presented elsewhere (S. C. Falco, M. Rose and D. Botstein, in preparation). The recombinants (class III) relevant here showed a stable Ura$^+$ Leu$^+$ phenotype.

Stability of the Ura$^+$ Leu$^+$ Phenotype in Haploid Class III Recombinants

Table 1 shows the stability of the Ura$^+$ and Leu$^+$ phenotypes of the original Leu$^+$ Ura$^-$ transformants obtained with pRB27 and pRB30 and several independent class III Ura$^+$ recombinants derived from each plasmid. The original transformants and the recombinants were subcloned nonselectively. Cells in the subclones were dispersed and replated nonselectively; when colonies had formed, these plates were then replica-plated to media lacking only uracil or leucine. In effect, individual cells in each subclone were assayed by this procedure for their Ura and Leu phenotypes. As shown in Table 1, the original transformants (which contain the plasmid in its autonomous form) segregate the plasmid (that is, lose the Leu$^+$

Table 1. Stability of Ura$^+$ and Leu$^+$ Phenotypes in Haploids during Unselected Growth

Plasmid	Recombinant	State of Plasmid	Total Number Tested	Phenotypes (Percentage of Total)			
				Ura$^+$ Leu$^+$	Ura$^+$ Leu$^-$	Ura$^-$ Leu$^+$	Ura$^-$ Leu$^-$
prB30 (*ORI*$^+$)	Parent (Ura$^-$)	Autonomous	250	0	0	73	27
	R30-3 (Ura$^+$)	Integrated	90	100	0	0	0
	R30-4 (Ura$^+$)	Integrated	53	100	0	0	0
	R30-8 (Ura$^+$)	Integrated	167	100	0	0	0
pRB27 (*ori*$^-$)	Parent (Ura$^-$)	Autonomous	154	0	0	26	74
	R27-7 (Ura$^+$)	Integrated	138	100	0	0	0
	R27-9 (Ura$^+$)	Integrated	41	100	0	0	0
	R27-17 (Ura$^+$)	Integrated	73	100	0	0	0

Single colonies grown nonselectively on YEPD solid medium were picked, diluted in water and spread on YPD plates. The colonies that grew were tested for their Ura and Leu phenotypes by replica-plating.

phenotype) frequently during mitotic growth. The class III Ura$^+$ recombinants maintain both Ura$^+$ and Leu$^+$ phenotypes stably during mitotic growth, suggesting that the entire plasmid might have integrated into a yeast chromosome, possibly as a result of a reciprocal crossover between the two mutant *ura3* alleles. Since class III recombinants were obtained with equal facility from transformants by using pRB27 and pRB30, such integration would be unrelated to the presence of the 2μ plasmid origin (*ORI*), which only pRB30 carries intact.

Physical Evidence for Integration of Plasmids Containing 2μ DNA

To show that the class III recombinants contain the entire plasmid (including the 2μ DNA) integrated into a yeast chromosome, we performed physical analysis of yeast genomic DNA from the recombinants by gel-transfer hybridization (Southern, 1975). Figure 2 shows a schematic diagram of the structures expected if plasmids pRB30 and pRB27 were integrated by homology at the *URA3* locus. Such an integration would generate restriction fragments (marked F1 and F2 in the case of pRB30 and F4 and F5 in the case of pRB27) representing junctions between plasmid and chromosomal sequences. These fragments should hybridize to radioactive probes containing pBR322 (vector) sequences or 2μ DNA sequences as indicated. Total yeast DNA extracted from strains carrying the putative integrated plasmids (and suitable control strains) was digested with restriction endonuclease into fragments that were separated by agarose gel electrophoresis, denatured in situ, transferred to nitrocellulose paper and hybridized to the appropriate radioactive DNA probe.

The results for stable Ura$^+$ Leu$^+$ recombinants derived from pRB30 (*ORI*$^+$) are shown in Figure 3A. When cut with the appropriate enzyme (Pvu II) for visualizing the fragments F1 and F2 (see Figure 2),

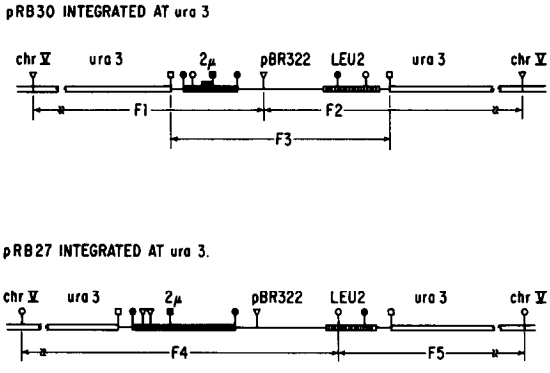

Figure 2. Diagram of Plasmids pRB30 and pRB27 Integrated at the *URA3* Locus on Chromosome V

(A) pRB30 integrated at *URA3*. F1 and F2: two new Pvu II restriction fragments. F1 contains pBR322 DNA and 2μ circle DNA. F2 contains pBR322 DNA but no 2μ circle DNA. Fragment F3, generated by digestion with Bam HI, includes all of pRB30 except for the *URA3* DNA.

(B) pRB27 integrated at *URA3*. F4 and F5: two new Hpa I restriction fragments. F4 contains most of the pBR322 DNA and 2μ circle DNA. F5 contains a small part of pBR322 (less than 10%) and no 2μ circle DNA. Restriction sites are as follows. (●) Eco RI. (○) Hpa I. (■) Xba I. (□) Bam HI. (▽) Pvu II.

three independent stable class III recombinants (the putative integrants) show both expected fragments when radioactive pBR322 is used as probe (lanes 3, 4 and 5 in Figure 3A), but show only F1, as expected, when radioactive 2μ DNA is used as probe (lanes 7, 8 and 9). Lanes 2 and 6 of Figure 3A show controls (yeast transformants containing autonomously replicating pRB30 plasmid); these contain very strong bands at positions corresponding to the positions expected for the plasmid itself and the various products of recombination between the plasmid and the resident 2μ circle. The junction fragments are not prominent in these control lanes, an observation much clearer in shorter exposures of these autoradiographs (not shown).

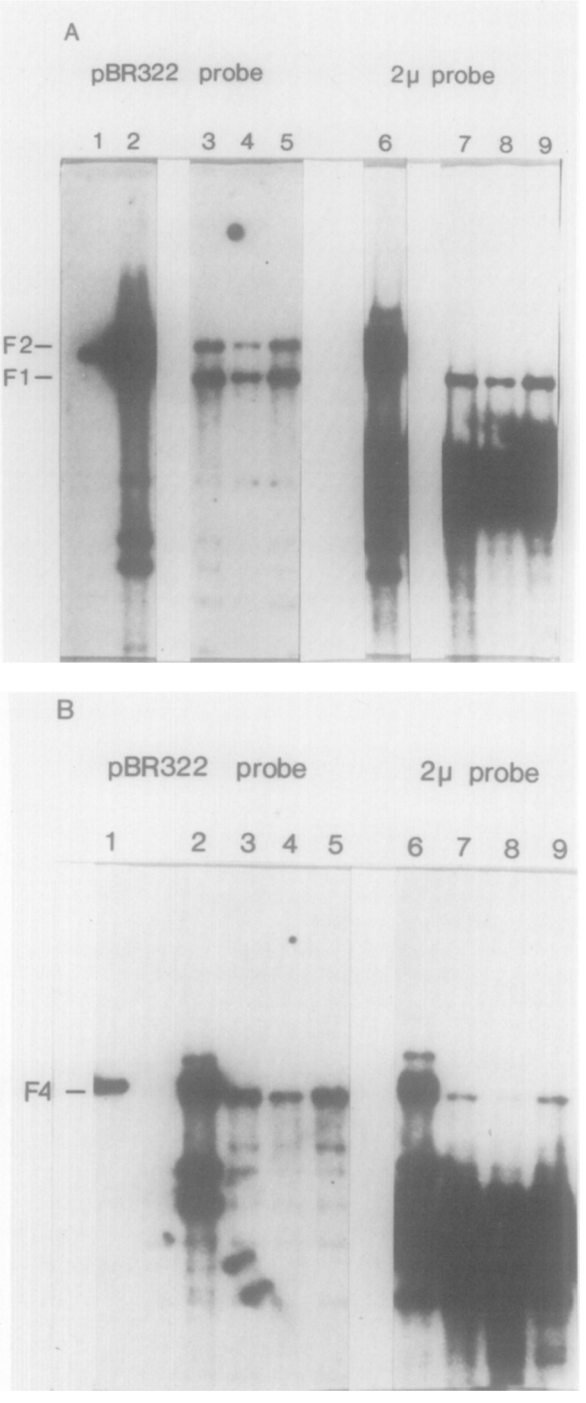

Figure 3. Physical Analysis of Class III Recombinants

Total genome DNA was obtained as described in Experimental Procedures. (A) DNA from pRB30-derived recombinants was digested by the restriction endonuclease Pvu II and fractionated according to size by electrophoresis on a 0.5% agarose gel. The DNA was transferred to nitrocellulose and hybridized to the indicated nick-translated probe DNA. (Lanes 1) pRB30; (lanes 2 and 6) parent transformant; (lanes 3 and 7) R30-3; (lanes 4 and 8) R30-4; (lanes 5 and 9) R30-8.

(B) DNA from pRB27-derived recombinants was digested with the restriction endonuclease Hpa I and fractionated according to size on a 0.5% agarose gel. The DNA was transferred to nitrocellulose and hybridized to the indicated nick-translated probe DNA. (Lane 1) pRB27; (lanes 2 and 6) parent transformant; (lanes 3 and 7) R27-7; (lanes 4 and 8) R27-9; (lanes 5 and 9) R27-17. Lanes not relevant to the experiment have been blocked out.

Similar results were obtained (Figure 3B) for stable Ura$^+$ Leu$^+$ derivatives of strains carrying pRB27 (*ORI*$^-$). The details are different: the enzyme used was Hpa I, which cleaves once in the plasmid, within the *LEU2* gene. As diagrammed in Figure 2, one junction fragment (F4) contains all the 2μ DNA sequences and most (92%) of the pBR322 sequences. This fragment is present in three independent class III recombinants with both probes (lanes 3, 4 and 5 for pBR322; lanes 7, 8 and 9 for 2μ DNA) and is not prominent in the control DNA (lanes 2 and 6) from strains containing the autonomously replicating plasmid.

Further analysis with different restriction enzymes (not shown) confirmed that the class III recombinants contain the entire plasmid integrated into the *URA3* locus on chromosome V of yeast.

Instability of the Ura$^+$ Leu$^+$ Phenotype in Heterozygous Diploids

To examine the genetic behavior of the integrated plasmids containing 2μ DNA, heterozygous diploids were constructed by mating haploid class III recombinants with *leu2*$^-$ *ura3*$^-$ strains of the opposite mating type. In the course of this analysis, the surprising observation was made that the Ura$^+$ and Leu$^+$ phenotypes were not nearly as stable as they had been in the haploid parents. This observation is illustrated in Table 2, which shows the stability of the phenotypes in diploids heterozygous for the integrated plasmids containing 2μ DNA. For each of the two plasmids (pRB30 and pRB27), the three independent recombinants shown above to have integrated the 2μ DNA-containing plasmid were tested and behave in the same way: after a period of unselected growth, about 25% of the diploids had lost both the Ura$^+$ and Leu$^+$ phenotypes. In no case were the Ura$^+$ and Leu$^+$ phenotypes lost independently.

To test the possibility that this effect is due not to heterozygous diploidy per se, but to the cell type, *MATa/MATa* and *MAT*α*/MAT*α diploids heterozygous for the integrated 2μ DNA-containing plasmid were also constructed. As shown in Table 2, the *a/a* and α/α diploids showed the same simultaneous loss at high frequency of the Leu$^+$ and Ura$^+$ phenotypes. Thus the cell type seems to have no influence, and the difference between the stability of haploids (Table 1) and heterozygous diploids (Table 2) carrying the same integrated plasmids appears to be a consequence of the diploid state as such.

As an additional control, diploids were constructed that were heterozygous for integrated plasmids carrying no 2μ DNA but otherwise identical to pRB30 and

Table 2. Stability of Ura$^+$ and Leu$^+$ Phenotypes in Diploids during Unselected Growth

Plasmid	Recombinant	Genotype at *MAT*	Total Number Tested	Phenotypes (Percentage of Total)			
				Ura$^+$ Leu$^+$	Ura$^+$ Leu$^-$	Ura$^-$ Leu$^+$	Ura$^-$ Leu$^-$
pRB30 (*ORI*$^+$)	Parent (Ura$^-$)	*a*/α	142	0	0	51	49
	R30-3 (Ura$^+$)	*a*/α	165	75	0	0	25
	R30-4 (Ura$^+$)	*a*/α	170	67	0	0	33
	R30-8 (Ura$^+$)	*a*/α	160	82	0	0	18
pRB27 (*ori*$^-$)	Parent (Ura$^-$)	*a*/α	70	0	0	1.4	98.6
	R27-7 (Ura$^+$)	*a*/α	117	73	0	0	27
	R27-9 (Ura$^+$)	*a*/α	159	78	0	0	22
	R27-17 (Ura$^+$)	*a*/α	122	75	0	0	25
	R27-7 (Ura$^+$)	*a*/*a*	91	83	0	0	17
	R27-7 (Ura$^+$)	α/α	86	48	0	0	52
pRB40 (pRB30 without 2μ DNA)		*a*/α	178	178	0	0	0
pRB42 (pRB27 without 2μ DNA)		*a*/α	166	166	0	0	0

The *MATa*/*MATα* diploids were all constructed by mating the parent recombinants (in strain DBY931) by strain DBY1042. The *MATa*/*MATa* diploid was obtained by selecting rare matings by complementing prototrophies between the recombinant R27-7 (in DBY931) and FY182. The *MATα*/*MATα* diploid was obtained analogously by crossing R27-7-23a (which is the R27-7 recombinant in a *MATα* background obtained from the cross R27-7 × DBY1042) with DBY746. Stability of the markers was determined as described in Table 1.

Table 3. Tetrad Analysis of Diploids Heterozygous for Integrated 2μ Plasmids

Recombinant Parent	Segregation of Leu$^+$ Ura$^+$:Leu$^-$ Ura$^-$			Linkage of Ura$^+$ and Leu$^+$ in 2:2 Tetrads	Linkage of Ura$^+$ Leu$^+$ to Can^r in 2:2 Tetrads		
	2:2	1:3	0:4		PD	NPD	T
R27-7a	16	1	2	100%	3	0	13
R27-7b	0	0	15				
R27-9	11	0	1	100%	3	0	8
R27-17	8	0	6	100%	3	0	5
R30-3	15	0	1	100%	8	0	7
R30-4	16	0	0	100%	8	0	8
R30-8	13	0	4	100%	6	0	7
Total	79	1	29	100%	31	0	48

Diploids were obtained by crossing the haploid recombinant parents by DBY1042. The diploids were purified and sporulated. Tetrads were dissected and the spore clones were analyzed for the indicated phenotypes. PD: parental ditype. NPD: nonparental ditype. T: tetrad type.

pRB27. As shown in the last two lines of Table 2, these constructions were perfectly stable, suggesting that it is the 2μ DNA in the integrated plasmids that is responsible for the instability in heterozygous diploids, and not something about the rest of the DNA in plasmids pRB30 and pRB27.

Genetic Consequences of Integration of 2μ circle DNA into a Chromosome

To study further the genetic nature of the instability of diploids heterozygous for an integrated plasmid containing 2μ DNA, tetrad analysis was carried out on sporulated cultures of the diploids described above. Most tetrads yielded two Ura$^+$ Leu$^+$ spores and two Ura$^-$ Leu$^-$ spores, as expected for chromosomally inherited markers, and in all cases these markers segregated together. No tetrads contained four Ura$^+$ Leu$^+$ spores. The results (Table 3) are consistent with the instability described above, since some of the tetrads yielded four Ura$^-$ Leu$^-$ spores. Occasionally (Table 3, line 2) all tetrads contained four Ura$^-$ Leu$^-$ spores, indicating that segregation had occurred before single-colony isolation.

Linkage to another marker on chromosome V (*CAN1*) could also be scored in the tetrads that segregated the Ura and Leu markers 2:2. As shown in Table 3, these data are consistent with the known linkage of *CAN1* to *URA3*.

In the course of the preceding analysis, it was observed that all the tetrads that had lost the *URA3*$^+$ and *LEU2*$^+$ markers had another characteristic in common—they all had become homozygous at the linked centromere-distal *CAN1* locus. To determine the relationship of the integrated 2μ DNA-containing plasmid to the events responsible for the homozygosis at the *CAN* locus, diploids were constructed (Table 4) that were heterozygous both for *can*^{r} and for the integrated 2μ DNA-containing (*ORI*$^+$ or *ORI*$^-$) plasmid. By using recombinants obtained from the crosses shown in Table 3, it was possible to have the *CAN*^{s} (dominant) allele both cis (that is, coupled) and *trans* (that is, in repulsion) to the integrated plasmid. These diploids were grown mitotically without selection for either marker and scored for Ura, Leu and Can phenotype. The results (Table 4) were as follows: when the dominant *CAN1* allele (CAN^s) was cis to the integrated plasmid, the frequency of phenotypically Can^r diploids was strikingly high (7% to 10%); when the *CAN*^{s} allele was on the other chromosome, the frequency was low (0.01% to 0.03%), roughly comparable to the frequency obtained in a control diploid containing no integrated 2μ DNA-containing plasmid. When such Ura$^-$ Leu$^-$ Can^r diploids were subjected to tetrad analysis (see above), there was reasonable spore viability; therefore, it seems likely that the Can^r phenotype observed in the experiment usually represented homozygosis at, and not physical loss of, the *CAN1* locus.

To determine whether the high degree of homozygosis observed at the *CAN1* locus is associated with loss of the integrated 2μ DNA-containing plasmid, many independent Ura$^-$ Leu$^-$ derivatives were isolated from diploids 2 and 3 (Table 4). These isolates were screened for their Can phenotype. In the case of diploid 2, 24/24 Ura$^-$ Leu$^-$ isolates were also Can^r, indicating homozygosity for the recessive allele. Diploid 3 yielded 22 Can^r derivatives among the 24 Ura$^-$ Leu$^-$ isolates. The two Can^s derivatives might be interesting exceptions from the general mechanism, but they might also represent a simple excision of the plasmid by a reversal of the integration by homologous recombination.

The reverse screening for coincidence was also performed. Independent Can^r derivatives of diploids 2 and 3 (Table 4) were screened for Ura and Leu phenotype. In each case, 24/24 Can^r derivatives were also Ura$^-$ Leu$^-$, indicating loss of the plasmid coincident with whatever event produced the homozygosis at *CAN1*.

These results suggest that the high frequency event responsible for the instability of the markers associated with integrated 2μ DNA-containing plasmid also is responsible for the effect on the instability of the centromere-distal linked marker. Even more striking, however, is the apparent directional specificity of the event; markers on the chromosome bearing the integrated plasmid become homozygous for the allele on the other homolog. All of these phenomena occurred at the same frequency regardless of whether the *ORI*$^+$ or *ORI*$^-$ 2μ DNA-containing plasmid was used.

One conceivable explanation for the directionality and coincidence of events at the locus of plasmid integration and the centromere-distal marker would be loss of the entire chromosome into which the plasmid is integrated. This kind of event would readily be observed in diploids, but not in haploids, where loss of the chromosome would be lethal. Even in diploids, however, chromosome loss would have to be followed quickly by restoration (endoreduplication?) of the full chromosome complement, since the tetrad data presented above (Table 3) showed frequent cases of 0:4 tetrads as opposed to the 0:2 (2 inviable) segregation expected for 2n-1 aneuploids.

To investigate this possibility further, diploids were constructed that were heterozygous for a 2μ DNA-containing plasmid integrated at the *URA3* locus, and also heterozygous at the *HIS1* locus (which lies on the other arm of chromosome V). As shown in Table 5, these diploids displayed the characteristic instability of the Ura$^+$ and Leu$^+$ phenotypes. In addition, a substantial fraction (up to about one third) of the Ura$^-$ Leu$^-$ segregants were also His$^-$. As was the case with Can^r, all the His$^-$ derivatives were Ura$^-$ Leu$^-$, but unlike the Can^r case, by no means all the Ura$^-$ Leu$^-$ derivatives were His$^-$. These results suggest that while information from the chromosome arm centromere-distal to the integrated 2μ DNA-containing plasmid virtually always is lost upon loss of the plasmid marker, the entire chromosome is not involved in the same way, since heterozygosity in the other chromosome arm is usually retained.

Table 4. Effect of Chromosomally Integrated 2μ DNA on Centromere Distal Marker

Diploid	Genetic Configuration	% Can^r Cells
1. (DBY931 × DBY1042)	*leu2 ura3 can*^{r} / *leu2 ura3 CAN*^{s}	0.004
2. (R27-7 × DBY1042)	*leu2 ura3* (Ura$^+$, Leu$^+$, 2μ Ori$^-$) *can*^{r} / *leu2 ura CAN*^{s}	0.03
3. (R30-3 × DBY1042)	*leu2 ura3* (Ura$^+$, Leu$^+$, 2μ Ori$^+$) *can*^{r} / *leu2 ura3 CAN*^{s}	0.01
4. (R27-7-23c × DBY931)	*leu2 ura3* (Ura$^+$, Leu$^+$, 2μ Ori$^-$) *CAN*^{s} / *leu2 ura3 can*^{r}	11.0
5. (R30-3-13d × DBY931)	*leu2 ura3* (Ura$^+$, Leu$^+$, 2μ Ori$^+$) *CAN*^{s} / *leu2 ura3 can*^{r}	6.8

Independent clones from each of the diploids were titered in the presence and absence of canavanine to determine the percentage of Can^r cells in the clone. R27-7-23c is a spore clone from the cross R27-7 × DBY1042. R30-3-13d is a spore clone from the cross R30-3 × DBY1042.

Table 5. Effect of Chromosomally Integrated 2μ DNA on a Marker on the Other Arm of the Chromosome

Integrated Plasmid	Recombinant	Total Number Tested	Phenotypes (Percentage of Total)			
			$His^+ Ura^+ Leu^+$	$His^+ Ura^- Leu^-$	$His^- Ura^+ Leu^+$	$His^- Ura^- Leu^-$
pRB30 (*ORI*$^+$)	R30-3	231	47	51	0	2.0
	R30-4	176	54	42	0	3.0
	R30-8	211	69	30	0	0.5
pBR27 (*ori*$^-$)	R27-7	208	63	28	0	8.0
	R27-9	169	42	48	0	10.0
	R27-17	158	57	35	0	8.0

The diploid strains were obtained by crossing the indicated recombinants with DBY1043. Stability was determined as described in Table 1. The diploid genotypes were:

$$\frac{a\ leu2\ ura3\ (URA3,\ LEU2,\ 2\mu)\ +}{\alpha\ leu2\ ura3 \qquad\qquad his1}.$$

The His^- Ura^- Leu^- segregants were subjected to tetrad analysis, with the result that lethal segregation (2 viable:2 lethal) was sometimes observed, as expected if the diploid was monosomic (2n-1). However, most isolates gave some tetrads with four viable spores. Further, some isolates appeared to give a mixture of asci with four viable and two viable spores, as if the population were mixed. Such a result might be expected if chromosome loss had occurred but was quickly followed by selection for restoration of chromosome balance during mitotic growth.

In all the experiments described thus far, the integrated 2μ DNA-containing plasmid was present on only one of the two chromosome V homologs. Strains homozygous for the integrated plasmids were constructed by using strains of opposite mating type generated by the experiment shown in Table 3. Diploids homozygous for integrated pRB27 (*ORI*$^-$) were constructed as well as diploids homozygous for integrated pRB30 (*ORI*$^+$). All the diploids were heterozygous at the *CAN1* locus. These diploids remained stably Ura^+ and Leu^+, as might be expected since they are homozygous for the integrated plasmid conferring these phenotypes. However, the instability at the *CAN* locus was still observed at high frequency (that is, 30% Can^r was not unusual). However, tetrad analysis in this case (not shown) showed that the directionality of the event was lost—as many tetrads were 4:0 *CAN*^{s}:*can*^{r} as were 0:4 *CAN*^{s}:*can*^{r}. Thus some of the instability of the chromosome associated with the integrated plasmids was retained, but the asymmetry or directionality was no longer observed. The presence of an active *ORI* made no difference; diploids homozygous for integrated pRB27 and pRB30 behaved identically.

All the observations described thus far involved 2μ DNA-containing plasmids integrated at the *URA3* locus on chromosome V. We have performed similar experiments at several other loci (*LEU2* on chromosome III [see also below] and *SUC2* on chromosome IX). In all cases, the results are similar to those shown for integration at *URA3*. Thus it appears that the unusual genetic properties we have observed are independent of the site of integration of 2μ-containing DNA into a yeast chromosome.

The Novel Genetic Properties of Diploids with Integrated 2μ DNA-Containing Plasmids Require an Intact *FLP* Gene on the Resident 2μ Circle

The 2μ DNA-containing plasmids pRB27 and pRB30 manifest unusual genetic properties when integrated into yeast chromosomes. The segments of 2μ DNA in these plasmids are nonoverlapping Eco RI restriction fragments, only one of which contains an intact *ORI* region, but both of which contain a complete copy of the 599 bp inverted repetition. Within this 599 bp segment is a site necessary for the rapid interconversion of the two forms of 2μ circle (Broach et al., 1982). Efficient interconversion requires an intact *FLP* gene and appears to be a form of site-specific recombination (Broach and Hicks, 1980; Broach et al., 1982).

Since the *FLP*-promoted site-specific recombination event is so efficient, it seemed possible that recombination between the integrated copy of the inverted repetition and one of the copies on the resident autonomous 2μ plasmid might frequently occur, with the result that an entire copy of the 2μ circle would become integrated into the chromosome in addition. This might be a very unstable structure, because *FLP*-promoted excision should also be frequent, which might somehow account for the genetic phenomena.

We attempted to detect the presence of an occasional additional 2μ circle DNA molecule integrated within the already integrated plasmid by gel-transfer hybridization (Southern, 1975), as described above. Digestion of pRB27 or pRB30 with Bam HI yields two DNA fragments. One of these includes pBR322 sequences, the *LEU2* gene and the 2μ DNA. The other is the 5.5 kb fragment carrying the *URA3* gene (see Figure 1). Digestion with Bam HI of the plasmid DNAs

integrated at the *URA3* locus yields the same fragments (one of these, containing the pBR322 sequences from integrated pRB30, is marked F3 in Figure 2). The 2μ circle contains no Bam HI targets. Therefore, integration of a whole additional 2μ plasmid would increase the size of the fragment carrying the pBR322 sequences by the length of the 2μ circle (6.3 kb).

When the total genomic DNA from strains carrying integrated pRB30 was cut with Bam HI and subjected to the gel-transfer hybridization procedure with the use of radioactive pBR322 as probe, the autoradiograph shown in Figure 4 was obtained. The dark band, 8.8 kb in size, was the expected length for the pBR322-containing Bam HI fragment of pRB30 (F3 in Figure 2). In this overexposed autoradiograph, however, a minor band (slightly larger than the 14.3 kb marker and corresponding to about 15.1 kb) is clearly visible. This band is of the size expected if an additional 2μ circle were integrated (8.8 + 6.3 = 15.1). Analogous experiments (not shown) with pRB27 gave similar results. It thus appears that integration of additional copies of 2μ circle does occur.

These results made it likely that *FLP*-promoted site-specific recombination might be involved in the genetic instability observed in diploids carrying an integrated plasmid containing 2μ DNA. To test this idea, several conditions must be met. First, plasmids must be integrated that are defective in *FLP* function. Second, strains must be available that lack 2μ plasmids and thereby cannot provide *FLP* function. Finally, a way must be found to introduce 2μ circles with wild-type or mutant *FLP* genes.

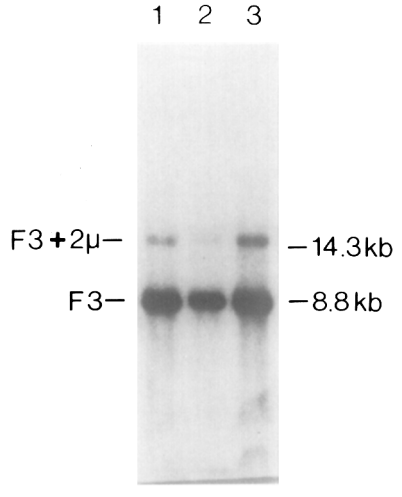

Figure 4. Integration of Entire 2μ Circle Plasmid into Chromosomally Integrated pRB30

Total genome DNA was obtained as described in Experimental Procedures. The DNA was digested with restriction endonuclease Bam HI and fractionated according to size by electrophoresis on a 0.5% agarose gel. The DNA was transferred to nitrocellulose and hybridized to nick-translated pRB322 DNA. The genomic DNA used was from recombinants R30-3 (lane 1), R30-4 (lane 2) and R30-8 (lane 3). DNA size standards derived from plasmid pRB30 were also run on the gel and are indicated. See text and Figure 2 for description of fragment F3.

The first condition is met by any plasmid containing only one of the Eco RI fragments of the 2μ plasmid, since lesions at one of the Eco RI sites have been shown to inactivate *FLP* (Broach and Hicks, 1980). The plasmids used in the experiments above are all *FLP*-defective; for the following experiments a simpler plasmid (CV7) was used that contains the same 2μ DNA fragment as pRB30 (that is, the small Eco RI fragment of the B form; therefore *ORI*$^+$) and that also contains the same *LEU2* fragment as the plasmids shown above. The second condition (a 2μ plasmid-free host) was obtained by using strain DC04, a *leu2*$^-$ derivative of Saccharomyces carlsbergensis, a type of yeast closely related to Saccharomyces cerevisiae and which lacks completely 2μ circle DNA (that is, it is *cir*^{0}; Broach and Hicks, 1980). The third condition can be achieved because *flp*$^-$ mutants of 2μ plasmids have recently been isolated (Broach et al., 1982) that can be introduced into strain DC04 by transformation and mating.

Strain DC04 was transformed to Leu$^+$ by using CV7 plasmid DNA. Even though CV7 is *ORI*$^+$, initial transformants are very unstable in this *cir*^{0} host; stable derivatives were found and shown to have the entire CV7 plasmid integrated at the *LEU2* locus by methods similar to those described above. This result indicates that there is no barrier to integration of plasmids containing 2μ DNA (including *ORI*$^+$) in strains lacking resident autonomous 2μ plasmids.

As already cited above, the integrated CV7 plasmid had genetic consequences, when heterozygous in diploids, which were identical to those observed in integration of 2μ DNA-containing plasmids at the *URA3* locus. When mated to a Saccharomyces cerevisiae strain that carries a normal complement of 2μ plasmids and has a mutation at the *HIS4* locus (which is centromere-distal to the plasmid integrated at *LEU2*), instability of both the Leu$^+$ phenotype (indicating loss of the plasmid) and the His$^+$ phenotype (indicating homozygosis at the distal marker) were observed (Table 6). This behavior extended also to markers on the other arm of chromosome III, since approximately 40% of the His$^-$ derivatives had also lost their nonmating phenotype and had become phenotypically *α*; this is consistent with homozygosis (or hemizygosis) at the *MAT* locus, which lies on the other arm of chromosome III from *LEU2* and *HIS4*.

To test whether Flp function is required for these instabilities, matings were carried out between the S. carlsbergensis strain DC04 carrying the CV7 plasmid integrated at *LEU2* and another *cir*^{0} *leu2*$^-$ S. carlsbergensis strain. No instability was observed. From this cross, recombinant derivatives with *a* mating type

Table 6. Effect of the 2μ *FLP* Gene on Stability of Markers on the Same Chromosome as Integrated 2μ Plasmid DNA

Genotype of Chromosome III in Diploid	Plasmid	Flp Phenotype	Total Number Tested	Mating Phenotype (%)			His Phenotype (%)	
				Nonmaters	a	α	His^+	His^-
I $\frac{a\ leu2\ (LEU2,\ 2\mu)\ +}{\alpha\ +\ \ \ \ his4}$	$2\mu^+$	Flp^+	1312	96	0	4[a]	89	11
II $\frac{a\ leu2}{\alpha\ leu2\ (LEU2,\ 2\mu)}$	CV21	Flp^+	2762	66	34	0		
	CV20	Flp^-	2533	100	0	0		
	CV21::Tn5-14	Flp^-	1596	100	0	0		

[a] All α maters were also His^-.

Strain DCO4, which lacks endogenous 2μ circles, (that is, cir^0), was transformed to Leu^+ by plasmid CV7, which replicates autonomously and yields unstable transformants. A stable Leu^+ derivative that is due to integration of the plasmid at the Leu2 locus on chromosome III was isolated. For experiment I, this recombinant was mated to strain K148 (cir^+). A culture of this diploid was grown for approximately ten generations in liquid culture (YEPD) from a single colony inoculum. The cells were then diluted and spread on rich solid media (YEPD). The colonies that grew were tested by replica-plating for their His phenotype and mating type. All α maters were His^-. For experiment II, a *MATα* derivative of the stable integrant of CV7 in DCO4 was obtained by crossing with strain SB1-1D (cir^0). This derivative was mated to strain DCO4 transformed by the indicated plasmids. These diploids were tested for mating type stability as described above.

were derived. These were crossed back to strain DCO4 derivatives carrying one of three plasmids (described elsewhere; Broach et al., 1982) containing all of the 2μ circle DNA inserted into a pBR322 derivative carrying the $LEU2^+$ gene. In plasmid CV20, the 2μ circle is split at the Eco RI site in the *FLP* region so that strains bearing CV20 are phenotypically Flp^-; in plasmid CV21, the 2μ circle is split at the other Eco RI site and strains bearing this plasmid are Flp^+. Plasmid CV21::Tn5-14 contains, in addition, a Tn5 transposon within the *FLP* gene, making strains bearing this plasmid phenotypically Flp^-.

The stability of Mat phenotype of these diploids is given in Table 6. The Leu phenotype could not be scored, since an active $LEU2^+$ copy is present both on the chromosome and on the CV20 or CV21 plasmid. It is clear from the data that both the Flp^- plasmids fail to show the instability characteristic of the Flp^+ control. Further, the asymmetry of the instability observed above when chromosome V is heterozygous for an integrated 2μ DNA-containing plasmid is again observed on chromosome III. It therefore is clear that Flp function is required for the instability associated with integration of 2μ DNA.

Discussion

The experiments described above have allowed us to show directly that 2μ plasmid DNA can become stably integrated into a yeast chromosome in haploid strains at several sites. Most of the experiments involved nonoverlapping fragments, each of which contain the 599 bp inverted repetition and which, between them, comprise the entire genetic complement of 2μ circle. The presence or absence of an intact origin for 2μ plasmid replication had no detectable influence in any of these cases. It was also possible to detect occasional integration of an additional copy of the intact 2μ circle DNA, within the already integrated plasmid.

Table 7. List of Yeast Strains

Strain	Genotype
DBY931	*a his4 leu2-1 leu2-112 met8-1 ura3-50 can1-101*
DBY1042	*α arg9 leu2-1 leu2-112 lys2 met8-1 ura3-50*
DBY1043	*α his1 leu2 ura3-50*
DBY746	*α his3 leu2-1 leu2-112 ura3-52 trp1-289*
FY182	*a his1 leu2-1 leu2-112 met8-1 ura3-50 can1-101*
DCO4	*a leu2-04 ade1* (2μcir^0) (Saccharomyces carlsbergensis)
K148	*α his4 lys2 trp met*
SB1-1D	*α leu2-04 ade1* (2μcir^0) (Saccharomyces carlsbergensis)

Previous investigations had indicated that, under normal conditions, little or no 2μ DNA is ever integrated into yeast chromosomes (Cameron et al., 1977; Sigurdson et al., 1981). The difference here is that the 2μ DNA that integrated was covalently attached to DNA with homology to chromosomal DNA, and a selection for recombination within the region of homology was used to detect these rare events. The genetic analysis confirmed that integration was always at a site of such homology. Kielland-Brandt et al. (1980) have also reported evidence for the chromosomal integration of 2μ circle DNA by homologous recombination.

Despite the apparent stability of integrated plasmids carrying 2μ DNA in haploid yeast, diploids heterozygous for such integrated plasmids showed a marked instability not only of the plasmid-associated genetic markers, but also of markers on the same chromosome. The instability of markers distal to the integrated

plasmid was extreme (up to 50% in a few generations of mitotic growth), while the instability of other markers on the other arm of the chromosome was less. Marker loss was tightly correlated to loss of the integrated plasmid.

The loss of information was strikingly asymmetrical in strains heterozygous for the integrated 2μ DNA-containing plasmid; the markers cis (that is, coupled) to the integrated plasmid were lost preferentially. In strains homozygous for the integrated plasmid, this asymmetry was no longer found, although distal markers still became homozygous at extremely high frequency. The instability in diploids was shown to require function of the 2μ plasmid *FLP* gene.

We can propose two kinds of models to account for the genetic properties of chromosomes into which 2μ DNA is inserted. The first kind of model envisions that the 2μ DNA provides a hot spot for mitotic recombination, perhaps owing to frequent breaks or lesions in the DNA catalyzed by the *FLP* gene in attempts to carry out site-specific recombination. The asymmetry of the events would have, in this kind of model, to be accounted for by some kind of directed gene conversion involving a whole chromosome arm; possibly the lesion caused by the Flp function results in a chromosome break or other loss of information requiring such a directed conversion.

A second, much more specific and more speculative model is shown in schematic form in Figure 5. The first step envisioned in this model is integration of a resident 2μ circle into the chromosome catalyzed by the high-frequency site-specific recombination system dependent upon the *FLP* gene. This is made possible by the presence in the chromosome of the inverted repetition sequence, which contains the substrate for site-specific recombination. We showed above that this integration seems to occur. As shown in Figure 5A, such an integration would result in the presence of three copies of the 599 bp repeated sequence—the two outer copies would be direct repetitions, while the central one would be in the opposite orientation.

The model then envisions replication of the chromosomes, followed by a *FLP*-promoted unequal sister chromatid exchange between sites on adjacent 599 bp repeated segments—that is, ones lying in inverted orientation relative to each other. Unequal sister chromatid exchange during mitotic growth has been observed in yeast in the ribosomal DNA cluster (Szostak and Wu, 1980). Unequal sister chromatid recombination has also been proposed by Kielland-Brandt et al. (1980) to account for a sequence rearrangement associated with chromosomally integrated 2μ circle.

The consequence of the unequal exchange event we propose (shown in Figure 5D) is the production of a dicentric chromosome and an acentric fragment. The acentric fragment (and the information on it) would be lost in subsequent growth and division. The dicentric might not disjoin at mitosis, resulting in a 2n-

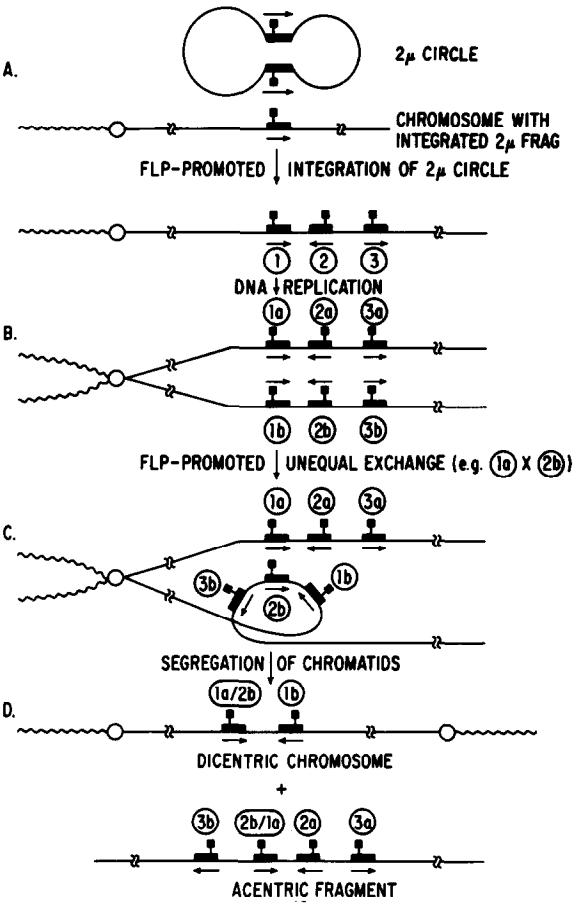

Figure 5. Model for Novel Genetic Events Associated with Integration of 2μ Circle DNA in a Yeast Chromosome

(A) *FLP*-promoted integration of resident 2μ circle plasmid into a chromosome by site-specific recombination with chromosomally integrated 2μ circle DNA. (B) Replication of chromosome. (C) *FLP*-promoted unequal exchange by site-specific recombination between inserted repetitions on sister chromatids. (D) Segregation of exchange products, yielding a dicentric chromosome and an acentric fragment.

1 monosomy (which was sometimes observed). Alternatively, the dicentric might break in the region between the two centromeres. We propose that the broken ends will be "recombinogenic" and "heal" themselves with use of the intact homolog as a template. This would explain the asymmetry of the homozygosis of distal markers—the information originally coupled to the integrated plasmid is lost on the acentric fragment so that reconstitution of an intact chromosome must necessarily use information from the homolog. Analogous "healing" processes have recently been proposed to explain the genetic consequences of some aberrant mating type switching events (McCusker and Haber, 1981).

Further work will have to be done in order to support or to disprove these speculative models. It does seem worth noting, however, that the second model incorporates many elements of the bridge-breakage-fusion cycle first described for maize by McClintock (1939, 1941, 1951). This resemblance, taken together with

the relatively natural explanation of our results thus far, makes the second model seem more reasonable and attractive.

We have developed several techniques based on the genetic properties of plasmids containing 2μ DNA. The ability of such plasmids to recombine with homologous regions on chromosomes provides a new way to carry out fine-structure mapping in yeast; deletion mutations on yeast chromosomes are relatively rare, whereas they can easily be made in vitro on plasmids. We have used such deletions to map point mutations on the chromosome (S. C. Falco, M. Rose and D. Botstein, manuscript in preparation).

A second, and probably more generally useful technique, takes advantage of the instability of markers on the same chromosome as an integrated plasmid containing 2μ DNA. If a DNA fragment of interest is incorporated into a plasmid containing 2μ DNA, and the resulting plasmid is integrated into the genome, then the chromosome from which the DNA of interest derives becomes unstable in diploids. If the diploids are suitably constructed with many recessive auxotrophic markers, the identity of the unstable chromosome can easily be ascertained. This method will also be described in detail elsewhere (S. C. Falco, D. Shortle, J. Thomas and D. Botstein, manuscript in preparation).

It may also be possible to exploit the properties of integrated 2μ plasmids in other ways. The frequency of chromosome loss may facilitate the construction of monosomic strains and the high frequency reciprocal site-specific recombination system could allow the generation of directed chromosomal rearrangements such as translocations, inversions, duplications and deletions.

Experimental Procedures

Culture Media

Solid and liquid media used for growth of yeast are described in Sherman et al. (1974). YEPD medium was used for nonselective growth. SD medium supplemented with appropriate nutrients was used for selective growth. Sporulation of diploid strains was performed on solid medium containing 2% potassium acetate, 0.1% glucose, 0.25% Bacto-yeast extract (Difco) and 1.5% Bacto-agar (Difco).

Yeast Transformation

Yeast strains were transformed by using the procedure of Hinnen et al. (1978) as stated or with the following modifications. The glusalase (Endo Labs) treatment of cells was performed in 1 M Sorbitol containing 1% β-mercaptoethanol and buffered to pH 5.8 with 0.1 M citrate buffer. The concentration of glusalase was 2%, and the spheroplasting treatment went for 3 hr at 30 °C.

Isolation of Ura$^+$ Plasmid × Chromosome Recombinants

Haploid transformants were grown as single colonies on selective solid medium in order to maintain the autonomously replicating plasmids. Single independent colonies were picked and spread on solid medium that lacked uracil to select Ura$^+$ recombinants.

Stability Measurements

Strains were grown nonselectively on YEPD solid medium. Single colonies were picked and diluted in water. Cells were then spread on YPD solid medium to yield many single colonies. The colonies were tested by replica-plating on SD medium supplemented with appropriate nutrients for the desired phenotypes.

Mating and Sporulation of Yeast

Strains to be mated were cross-streaked on YPD solid medium and grown at 30°C overnight. Diploids were then selected by replica-plating onto minimal solid medium (SD) supplemented with the nutrients required for the particular mating. Diploids were then single-colony purified on YEPD solid medium and transferred to sporulation medium. After 3 days at 30°C, asci were digested in 2% glusalase and spores were separated by micromanipulation.

DNA Preparation

Plasmid DNA was prepared from bacteria by equilibrium density sedimentation in a cesium chloride–ethidium bromide gradient as described by Davis et al. (1980). Total genomic DNA from yeast was prepared as described by Carlson and Botstein (1982), with the following modification. Before the final ethanol precipitation of the DNA, a chloroform-isoamyl alcohol extraction was performed. To the DNA resuspension (0.25 ml), an equal volume of a mixture of chloroform and isoamyl alcohol (24:1; v/v) was added. The solutions were mixed until a homogeneous white emulsion was formed. The phases were then separated by centrifugation and the upper aqueous phase containing the DNA was removed.

Physical Analysis of Heat Genomic DNA

Agarose gel electrophoresis, transfer of DNA to nitrocellulose, α-^{32}P-labeling of DNA for use as a hybridization probe by nick translation and hybridization of DNA were all performed as described by Davis et al. (1980).

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References

Bak, A., Christiansen, C. and Christiansen, G. (1972). Biochim. Biophys. Acta *269*, 527–530.

Beggs, J. D. (1978). Transformation of yeast by a replicating hybrid plasmid. Nature *275*, 104–109.

Beggs, J. D., Guerineau, M. and Atkins, J. F. (1976). A map of the restriction targets in yeast 2μ plasmid DNA cloned on bacteriophage lambda. Mol. Gen. Genet. *148*, 287–294.

Bolivar, R., Rodriguez, R. L., Greene, P. J., Betlachk, M. C., Heyneker, H. L., Boyer, H. W., Crosa, J. H. and Falkow, S. (1977). Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene *2*, 95–113.

Botstein, D., Falco, S. C., Stewart, S., Brennan, M., Scherer, S., Stinchcomb, D. T., Struhl, K. and Davis, R. (1979). Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. Gene *8*, 17–24.

Broach, J. R. (1981). The yeast plasmid 2μ circle. In the Molecular Biology of the Yeast Saccharomyces, J. Strathern, E. Jones and J. Broach, eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 445–470.

Broach, J. R. and Hicks, J. B. (1980). Replication and recombination

functions associated with the yeast plasmid, 2μ circle. Cell *21*, 501–508.

Broach, J. R., Atkins, J. F., McGill, C. and Chow. L. (1979). Identification and mapping of the transcriptional and translational products of the yeast plasmid, 2μ circle. Cell *16*, 827–839.

Broach, J. R., Guarascio, V. R. and Jayaram, M. (1982). Recombination within the yeast plasmid 2μ circle is site-specific. Cell *29*, 227–234.

Cameron, J. R., Philippson, P. and Davis, R. W. (1977). Analysis of chromosomal integration and deletions of yeast plasmids. Nucl. Acids Res. *4*, 1429–1448.

Carlson, M. and Botstein, D. (1982). Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell *28*, 145–154.

Clark-Walker, G. D. and Miklos, G. L. G. (1974). Localization and quantification of circular DNA in yeast. Eur. J. Biochem. *41*, 359–365.

Davis, R. W., Botstein, D. and Roth, J. R. (1980). Advanced Bacterial Genetics: A Manual for Genetic Engineering (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Gubbins, E. J., Newlon, C. S., Kann, M. D. and Donelson, J. E. (1977). Sequence organization and expression of a yeast plasmid. Gene *1*, 185–207.

Guerineau, M., Grandchamp, C. and Slonimski, P. (1976). Circular DNA of a yeast episome with two inverted repeats: structural analysis of a restriction enzyme and electron microscopy. Proc. Nat. Acad. Sci. USA *73*, 3030–3034.

Hartley, J. L. and Donelson, J. E. (1980). Nucleotide sequence of the yeast plasmid. Nature *286*, 860–864.

Hinnen, A., Hicks, J. B. and Fink, G. R. (1978). Transformation of yeast. Proc. Nat. Acad. Sci. USA *75*, 1929–1933.

Hollenberg, C. P., Degelman, A., Kusterman-Kuhn, B. and Royer, H. D. (1976). Characterization of 2-μm DNA of *Saccharomyces cerevisiae* by restriction fragment analysis and integration in an *Escherichia coli* plasmid. Proc. Nat. Acad. Sci. USA *73*, 2072–2076.

Kielland-Brandt, M. C., Nilsson-Tillgren, T., Litske Peterson, J. R. and Holmberg, S. (1980). Transformation in yeast without the involvement of bacterial plasmids. In Molecular Genetics in Yeast, Alfred Benzon Symposium *16*, D. von Wettstein, J. Friis, M. Kielland-Brandt and A. Stenderup, eds. (Copenhagen: Munksgaard), pp. 370–382.

Livingston, D. M. (1977). Inheritance of 2 μm DNA plasmid from *Saccharomyces*. Genetics *86*, 73–84.

Livingston, D. M. and Hahne, S. (1979). Isolation of condensed intracellular form of the 2 μm DNA plasmid of *Saccharomyces cerevisiae*. Proc. Nat. Acad. Sci. USA *76*, 3727–3731.

Livingston, D. M. and Klein, H. L. (1977). Deoxyribonucleic acid sequence organization of a yeast plasmid. J. Bacteriol. *129*, 472–481.

McClintock, B. (1939). The behavior of successive nuclear divisions of a chromosome broken at meiosis. Proc. Nat. Acad. Sci. USA *25*, 405–416.

McClintock, B. (1941). The stability of broken ends of chromosomes in *Zea maize*. Genetics *26*, 234–282.

McClintock, B. (1951). Chromosome organization and genic expression. Cold Spring Harbor Symp. Quant. Biol. *16*, 13–47.

McCusker, J. H. and Haber, J. E. (1981). Evidence of chromosomal breaks near the mating type locus of *S. cerevisiae* that accompany MAT × MAT matings. Genetics *99*, 383–403.

Nelson, R. G. and Fangman, W. L. (1979). Nucleosome organization of the yeast 2 μm DNA plasmid: a eukaryotic minichromosome. Proc. Nat. Acad. Sci. USA *76*, 6515–6519.

Rose, M., Casadaban, M. J. and Botstein, D. (1981). Yeast genes fused to β-galactosidase in *Escherichia coli* can be expressed normally in yeast. Proc. Nat. Acad. Sci. USA *78*, 2460–2464.

Sherman, F., Fink, G. R. and Lawrence, C. W. (1974). Methods in Yeast Genetics (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Sigurdson, D. C., Gaarder, M. E. and Livingston, D. M. (1981). Characterization of the transmission during cytoductant formation of the 2μm DNA plasmid from *Saccharomyces*. Mol. Gen. Genet. *183*, 59–65.

Southern, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. *98*, 503–517.

Szostak, J. W. and Wu, R. (1980) Unequal crossing over in the ribosomal DNA of *Saccharomyces cerevisiae*. Nature *284*, 426–430.

Taketo, M., Jazwinski, S. M. and Edelman, G. M. (1980). Association of the 2μm DNA plasmid with yeast folded chromosome. Proc. Nat. Acad. Sci. USA *77*, 3144–3148.

Note Added in Proof

In Figure 1 a Pvu II site is incorrectly indicated in the A_p^R region of pRB27 and pRB30. The site should appear approximately one third of the distance clockwise from the end of the LEU2$^+$ insert to the beginning of the 2μ insert.