HOMOLOGOUS RECOMBINATION BETWEEN EPISOMAL PLASMIDS AND CHROMOSOMES IN YEAST

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ABSTRACT

We have observed genetic recombination between *ura3*$^-$ mutations (among them extensive deletions) carried on "episomal" (*i.e.*, 2μ DNA-containing) plasmids and other *ura3*$^-$ alleles present at the normal chromosomal *URA3* locus. The recombination frequency found was comparable to the level observed for classical mitotic recombination but was relatively insensitive to sunlamp radiation, which strongly stimulates mitotic recombination. Three equally frequent classes could be distinguished among the recombinants. Two of these are the apparent result of gene conversions (or double crossovers) which leave the *URA3*$^+$ allele on the chromosome (class I) or on the plasmid (class II). The third class is apparently due to a single crossover that results in the integration of the plasmid into a chromosome. Plasmid-chromosome recombination can be useful in fine structure genetic mapping, since recombination between a chromosomal point mutation and a plasmid-borne deletion mutation only 25 base pairs distant was easily detected.

UNAMBIGUOUS demonstration of transformation of yeast (*Saccharomyces cerevisiae*) with DNA was first accomplished using plasmid DNAs carrying cloned yeast genes (HINNEN, HICKS and FINK 1978) that had first been amplified in *E. coli*. The transformants examined had evidently resulted from recombination events between the transforming DNA molecules and the homologous sequences on the yeast chromosomes. Soon thereafter, it was found that inclusion in the transforming plasmid of segments of DNA from the natural yeast plasmid (2μ circle) could enhance the frequency of transformation more than 1000-fold (BEGGS 1978). Physical and genetic experiments showed that such transformants contained autonomously replicating molecules (BEGGS 1978; STRUHL *et al.* 1979). Further study showed that the autonomous replication of such plasmids is essential to the high transformation frequency: plasmids that lacked the 2μ circle origin of DNA replication could transform at high frequency only if the recipients contained resident 2μ circles with which the incoming plasmids recombine freely *via* a site-specific recombination system (BROACH and HICKS 1980; BROACH, GUARASCIO and JAYARAM 1982). Recombination between the transforming plasmid and homologous sequences on the

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chromosome apparently played no role in DNA transformation with such plasmids, which were called YEp, for *y*east *e*pisomal *p*lasmids.

Homologous recombination between repeated genes has been demonstrated in yeast, using recombinant DNA techniques, in several instances. Reciprocal and nonreciprocal crossovers have been observed between genes repeated on nonhomologous chromosomes (SCHERER and DAVIS 1980; ERNST, STEWART and SHERMAN 1981; MIKUS and PETES 1982). Gene conversion has been observed between two alleles of a gene present as a duplication on the same chromosome (KLEIN and PETES 1981; JACKSON and FINK 1981). In addition, sister strand exchange occurs among the tandemly repeated ribosomal RNA genes (PETES 1980; SZOSTAK and WU 1980).

In this report we use mutant alleles of the yeast *URA3* gene carried on YEp vectors to investigate recombination between episomal plasmids and yeast chromosomes. We show that recombination between such plasmids and chromosomes is easily detected. We identify and characterize three distinct classes of recombinant progeny which provide evidence that both reciprocal and nonreciprocal plasmid-chromosome crossovers occur. Finally, we demonstrate the use of plasmid-chromosome recombination in fine structure deletion mapping of a yeast gene.

MATERIALS AND METHODS

Yeast strains: The strains used in this report are shown in Table 1. The *ura3* mutant alleles were obtained from F. LACROUTE. New strains were constructed using standard genetic techniques (SHERMAN, FINK and LAWRENCE 1974).

Culture media: Solid and liquid media used for growth of yeast are described in SHERMAN, FINK and LAWRENCE (1974). YEPD medium was used for nonselective growth. Sporulation of diploid strains was done 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 using the procedure of HINNEN, HICKS and FINK (1978) with the following modification. The glusulase (Endo Labs) treatment of cells was done in 1 M sorbitol containing 1% β-mercaptoethanol and buffered to pH 5.8 with 0.1 M citrate buffer. The concentration of glusulase was 2% and incubation was for 3 hr at 30°.

Determination of recombination frequencies: Strains transformed by autonomously replicating plasmids were grown as single colonies on selective solid medium to maintain the plasmids. Single independent colonies were picked and dispersed in water. Individual colonies were titered for a total number of Leu^+ cells (*i.e.*, cells that retain the plasmid) and Ura^+ cells. In crosses in which amber mutations were involved, putative Ura^+ recombinants were further characterized to identify pseudorevertants resulting from nonsense suppressors. This was accomplished by replica plating the Ura^+ colonies onto plates lacking methionine. Clones that grew on these plates, and thus coincidentally became Ura^+ and Met^+, were presumed to be due to a nonsense suppressor that suppressed both the *ura3* amber mutation and the *met8-1* amber mutation. The recombination frequency is expressed as the number of Ura^+ Met^- cells divided by the total number of Leu^+ cells and is the average of at least five independent measurements for each cross.

Stimulation of mitotic recombination by sunlamp irradiation was based on the method of LAWRENCE and CHRISTENSEN (1974) for fine structure mapping in yeast. Approximately 1×10^6 viable cells were spread on solid medium lacking uracil and exposed in closed plastic Petri dishes to 6 min of sunlamp radiation under four FS20T12 fluorescent sunlamps (Westinghouse). Plates were then incubated in the dark at 30°, and Ura^+ colonies were scored. Under these conditions, no significant decrease in cell viability was observed for either haploid or diploid strains.

Stability measurements: Strains were grown nonselectively on YEPD solid medium. Single colonies were picked and diluted in water. Cells were then spread on YEPD solid medium to yield many

TABLE 1

List of strains

Strain	Genotype	Source
FY188	**a** *his4-159 leu2-3 leu2-112 met8-1 ura3-3*	This work
FY189	**a** *his4-159 leu2-3 leu2-112 met8-1 ura3-18*	This work
DBY689	**a** *leu2-3 leu2-112 ura3-50 can1*	BOTSTEIN, Massachusetts Institute of Technology (MIT)
DBY745	**a** *ade1-100 leu2-3 leu2-112 ura3-52*	BOTSTEIN, MIT
DBY931	**a** *his4 leu2-3 leu2-112 met8-1 ura-50 can1-101*	This work
FD33	**a** *his4 +* / *α + leu2* — *ura3-3 +* / *ura3-18 can1*	This work
FD34	**a** *his4 +* / *α + leu2* — *ura3-3 +* / *ura3-3 can1*	This work
FD35	**a** *his4 +* / *α + leu2* — *ura3-18 +* / *ura3-18 +*	This work
FD36	**a** *his4 leu2* / *α + leu2* — *ura3-50 +* / *ura3-3 can1*	This work
FD39	**a** *his4 leu2* / *α + leu2* — *ura3-50 +* / *ura3-18 can1*	This work
FD50	**a** *his4 leu2* / *α + leu2* — *+ ura3-50 can1* / *arg9 ura3-50 +* — *met8-1 +* / *met8-1 lys2*	This work

single colonies. The colonies were tested by replica plating on SD medium supplemented with appropriate nutrients for the desired phenotypes.

DNA preparation: Plasmid DNA was prepared from bacteria by equilibrium density sedimentation in a CsCl-ethidium bromide gradient as described (DAVIS, BOTSTEIN and ROTH 1980).

RESULTS

Recombination between yeast genes carried on YEp plasmids (*i.e.*, plasmids containing 2μ circle DNA) and their homologues on yeast chromosomes was studied using a set of plasmids whose structure is shown in Figure 1. Each of the plasmids is derived from the common bacterial cloning vector pBR322 (BOLIVAR *et al.* 1977) and contains three fragments of yeast DNA: first, a 2.3-kb fragment carrying the *LEU2* gene; second, a 5.5-kb fragment containing a mutant allele (*ura3-3* or *ura3-18*) of the *URA3* gene; and third, a portion of the 2μ circle DNA. Such plasmids are maintained reasonably stably as autonomously replicating DNA molecules when introduced in yeast strains already containing intact 2μ circles. The resident 2μ circles provide diffusible replication functions; since the incoming plasmids freely recombine with the intact resident plasmids *via* a site-specific recombination system (BEGGS 1978; STRUHL *et al.* 1979; BROACH and HICKS 1980; BROACH, GUARASCIO and JAYARAM 1982), YEp plasmids are replicated autonomously whether or not they carry the 2μ origin of replication.

Recombination between point mutations in haploids and diploids: The YEp plasmids (Figure 1) were introduced by DNA transformation into *leu2*$^-$ haploid yeast strains also carrying the mutant *URA3* alleles *ura3-3* or *ura3-18* selecting for the function of the *LEU2* gene. The *ura3-3* and *ura3-18* alleles are amber

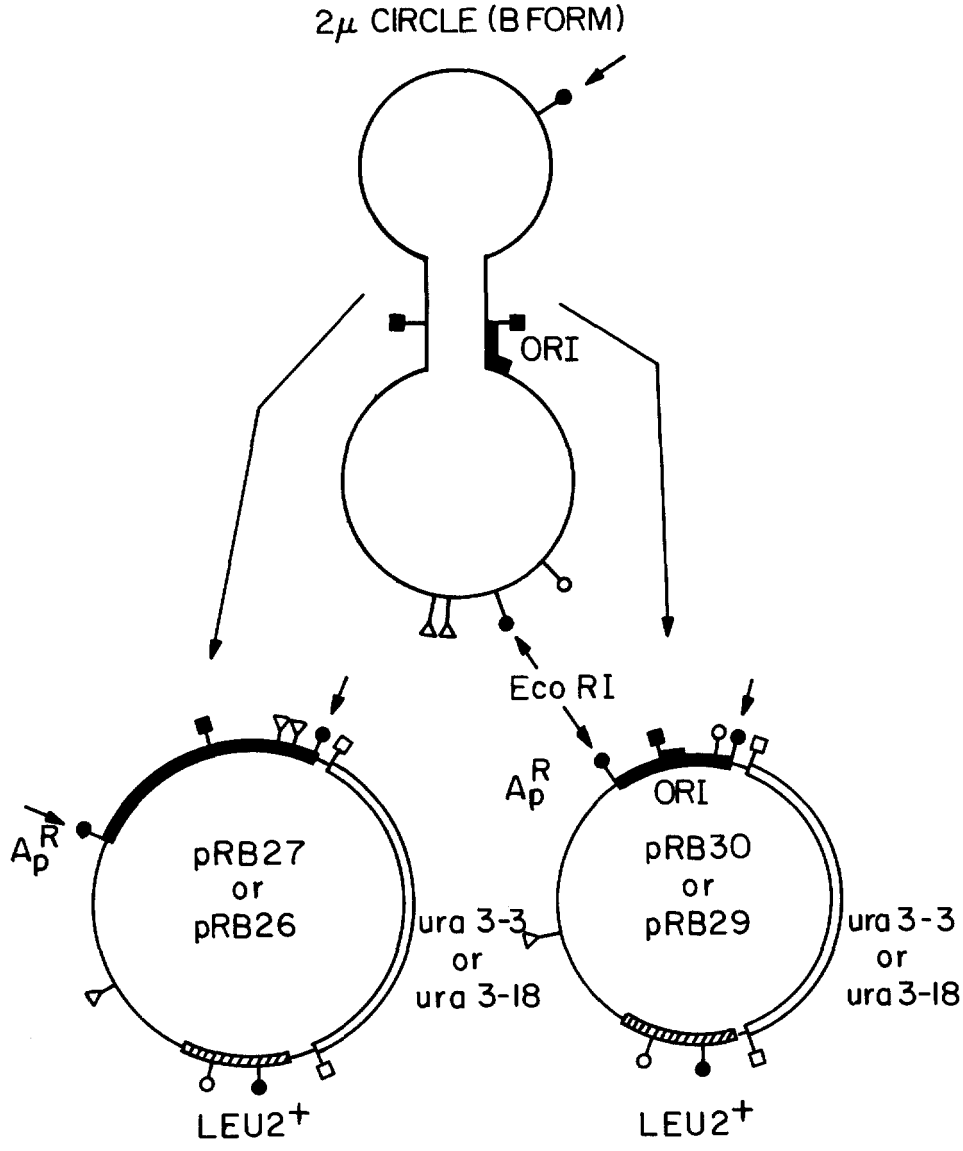

FIGURE 1.—Structure of plasmids used in this work is shown relative to the B form of the 2μ circle DNA. Each of the plasmids has one of the *ura3* mutations as indicated. Details of the construction are given in FALCO *et al.* (1982). Symbols for restriction cleavage sites are as follows. (●): *Eco*RI; (♀): *Hpa*I; (■): *Xba*I; (□): *Bam*HI; (▽): *Pvu*II.

mutations near the beginning and end, respectively, of the *URA3* coding sequence (LOSSON and LACROUTE 1979; ROSE 1982; ROSE, GRISAFI and BOTSTEIN 1983). As expected, the Leu^+ transformants were all still Ura^-, since both the plasmid and the chromosome carry mutant *URA3* genes. When the lesions in the *URA3* gene were not the same in the plasmid and the chromosomal genes (these are called hereafter "heteroallelic transformants"), recombination could

be followed by observing the appearance of Ura$^+$ derivatives. Controls for the frequency of reversion consisted of strains that carried the same mutation on the plasmid and the chromosome ("homoallelic transformants") as well as strains that had never been transformed. Two kinds of reversion are possible because both the *ura3*$^-$ alleles used are amber mutations; therefore, the strains used contain an additional amber mutation (*met8-1*) that was used to detect amber suppressors.

The actual measurements of recombination were carried out simply by isolating subclones of each transformant on medium that lacks leucine and, thus, selects maintenance of the plasmid. Colonies were suspended in water and replated on medium lacking uracil as well as leucine and comparing the number obtained with the number found when uracil is provided. Ura$^+$ colonies were also tested for the Met$^+$ phenotype (indicating appearance of a suppressor); Met$^+$ Ura$^+$ revertants were the great majority (95% or more) of total Ura$^+$ derivatives in the case of homoallelic transformants or untransformed strains, whereas they were a small minority (10% or less) in the case of heteroallelic transformants. The number of Met$^+$ Ura$^+$ suppressor revertants was always subtracted from the total Ura$^+$ colonies observed.

The results of a typical experiment are shown in Table 2. It is clear that the heteroallelic transformants yielded many more Ura$^+$ derivatives than the homoallelic transformants; the difference is about 20-fold and does not depend upon which of the alleles is plasmid borne in the heteroallelic cases. This increase in uracil-independent derivatives is attributable to recombination between the plasmid and the chromosome since the only difference between the homoallelic and heteroallelic transformants is the position and identity of the *ura3*$^-$ mutations.

To place the frequency of recombination observed between the plasmid and chromosome in haploids into some context, Table 2 also shows an estimate of the frequency of spontaneous mitotic recombination between the same two *ura3*$^-$ alleles. This estimate was obtained by simply comparing the frequency of Ura$^+$ in heteroallelic and homoalleleic diploids. It is clear from Table 2 that the frequency of spontaneous mitotic recombination between the two *ura3*$^-$ alleles was very similar to that found between the same alleles in the heteroallelic transformants. Direct quantitative comparison of frequencies must be made with caution, since the diploids we used are heterozygous at the *MAT* locus, which generally increases the frequency of mitotic recombination.

Further characterization of plasmid-chromosome recombination was carried out using transformants of the haploid strain DBY931, which carries the nonreverting *ura3-50* mutation, which is located between *ura3-3* and *ura3-18* but much more closely linked to the latter (LOSSON and LACROUTE 1979). In addition to the YEp plasmids pRB30 and pRB29, two additional YEp plasmids [pRB27(*ura3-3*) and pRB26 (*ura3-18*)] were used. These differ from pRB30 and pRB29 only in that they have a different fragment of the 2μ circle DNA which does not contain the plasmid origin of replication. Table 3 shows that recombination between plasmid and chromosome was again observed at frequencies that differ depending upon the markers used but independent of the

TABLE 2

Plasmid-chromosome recombination

Strain		URA3 alleles		Ura$^+$ frequencey ($\times 10^{-7}$)
Host	Plasmid	Chromosome	Plasmid	
FY188	None	*ura3-3*		<5.0
FY188	pRB30	*ura3-3*	*ura3-3*	5.0
FY188	pRB29	*ura3-3*	*ura3-18*	110.0
FY189	None	*ura3-18*		<5.0
FY189	pRB29	*ura3-18*	*ura3-18*	<5.0
FY189	pRB30	*ura3-18*	*ura3-3*	70.0
FD34	None	*ura3-3/ura3-3*		<5.0
FD35	None	*ura3-18/ura3-18*		<5.0
FD33	None	*ura3-3/ura3-18*		50.0

TABLE 3

Further characterization of plasmid-chromosome recombination

Strain		URA3 alleles		*Ura*$^+$ frequency ($\times 10^{-7}$)		
Host	Plasmid	Chromosome	Plasmid	Sponta-neous	Sunlamp	Stimulation factor
DBY931	None	*ura3-50*		<0.1	<0.1	
DBY931	pRB30	*ura3-50*	*ura3-3*	210	700	3.3
DBY931	pRB27 (*ori*$^-$)	*ura3-50*	*ura3-3*	100	200	2.0
DBY931	pRB29	*ura3-50*	*ura3-18*	32	110	3.3
DBY931	pRB26 (*ori*$^-$)	*ura3-50*	*ura3-18*	38	94	2.5
FD36	None	*ura3-50/ura3-3*		80	1100	13
FD39	None	*ura3-50/ura3-18*		<5	60	>10
FD50	None	*ura3-50/ura3-50*		<0.1	<0.1	
FD50	pRB30	*ura3-50/ura3-50*	*ura3-3*	420	1300	2.5
FD50	pRB29	*ura3-50/ura3-50*	*ura3-18*	71	200	2.8

presence of the 2μ circle origin of DNA replication. When compared with the frequency of mitotic recombination (also shown in Table 3) for the same allele pairs, the results were comparable in the case of crosses between *ura3-50* and *ura3-3*; however, in the case of the crosses between *ura3-50* and *ura3-18*, the mitotic recombination was undetectably low (less than 5×10^{-7}), whereas the corresponding heteroallelic transformants yielded substantial numbers of recombinants. The spontaneous recombination frequencies between plasmid and chromosome are, thus, consistent with the genetic distances determined by other genetic methods, although the degree of decrease of recombination with decreasing distance must be less. These results suggest that plasmids might offer advantages for mapping over short genetic distances.

Table 3 also shows the results of spontaneous recombination between plas-

mid and chromosomes in strain FD50, a diploid homozygous for the *ura3-50* mutation. FD50 was transformed with pRB30 and pRB29, and the frequency of Ura$^+$ recombinants was measured as before. The frequencies of recombination found were about twice those found in the corresponding haploids; otherwise, there seems to be no difference between the cell types.

Finally, we examined the effect of sunlamp radiation upon plasmid-chromosome recombination. These results are also shown in Table 3. As reported previously (LAWRENCE and CHRISTENSEN 1974), sunlamp radiation stimulates mitotic recombination between closely linked markers. This result was reproduced in the mitotic crosses in Table 3, where factors of increase of more than tenfold relative to the spontaneous rate were found. However, the stimulation of recombination between plasmid and chromosome by the same doses of sunlamp radiation was much more modest, only about threefold. This result is reproduced at other doses as well (not shown). The weakness of the stimulation was independent of the cell type (haploid or diploid) and the presence on the plasmid of the 2μ circle origin of DNA replication. In other experiments (not shown) irradiation with standard (260 nm) UV light resulted in a similarly weak stimulation of recombination between plasmid and chromosome.

Three classes of recombinants between plasmids and chromosomes: Strain DBY931 (*ura3-50 leu2*) was transformed with plasmid pRB30 or pRB29 (both plasmids carry *ura3-3*; see Table 3) selecting Leu$^+$. Independent Ura$^+$ recombinants that arose were selected and subcloned, maintaining selection for the Ura$^+$, but not the Leu$^+$, phenotype. Cells from the subclones were dispersed in water and replated nonselectively on rich medium. Colonies that formed on the rich plates were replica plated to appropriate minimal media in order to score the Leu$^+$ and Ura$^+$ phenotypes independently of each other. Three classes of recombinants could be distinguished, based on the stability and segregation of the Ura$^+$ and Leu$^+$ phenotypes, as shown in Table 4.

Class I recombinants showed no correlation between the Ura$^+$ and Leu$^+$ phenotypes. In many of them, most or all cells in the subclones were Leu$^-$, indicating that the original transforming plasmid had already been lost. Physical analysis (not shown) by gel-transfer hybridization (SOUTHERN 1975) detected no plasmid sequences in such Ura$^+$Leu$^-$ segregants. In contrast, the Ura$^+$ phenotype in class I recombinants was completely stable, indicating that the chromosomal gene had become *URA*$^+$. Meiotic analysis confirms this, since the Ura$^+$ phenotype of class I recombinants segregates 2:2 in a cross with a *ura3*$^-$ strain and 4:0 in a cross with a *URA3*$^+$ strain (not shown). Class I recombinants are, thus, easily explained by a double crossover (or, more likely, gene conversion) event that results in the replacement of the chromosomal *ura3-50* allele with wild-type information from the plasmid. The recovery of class I recombinants lacking the plasmid provides additional evidence for plasmid-chromosome recombination, since the *ura3-50* allele has never been observed to revert.

Class II recombinants showed complete correlation of the Ura$^+$ and Leu$^+$ phenotypes, but both phenotypes were unstably maintained in the cells of the subclones. This indicates that both markers are on plasmids. Furthermore,

TABLE 4

Charcterization of plasmid-chromosome recombinants

Plasmid	Ura$^+$ isolate	No. tested	Phenotype: % of total				Class
			Ura$^+$ Leu$^+$	Ura$^+$ Leu$^-$	Ura$^-$ Leu$^+$	Ura$^-$ Leu$^-$	
pRB27 (*ori*$^-$)	1	220	0	100	0	0	I
	2	70	0	100	0	0	I
	3	126	21	79	0	0	I
	5	62	0	100	0	0	I
	7	99	0	100	0	0	I
	10	68	0	100	0	0	I
	14	52	0	100	0	0	I
	18	51	35	65	0	0	I
	4	100	26	0	0	74	II
	9	54	26	0	0	74	II
	11	77	35	0	0	65	II
	12	95	21	0	0	79	II
	13	104	37	0	0	63	II
	15	59	60	0	0	40	II
	17	82	52	0	0	48	II
	6	138	100	0	0	0	III
	8	41	100	0	0	0	III
	16	73	100	0	0	0	III
pRB30 (*ORI*$^+$)	2	139	0	100	0	0	I
	11	34	32	68	0	0	I
	18	53	0	100	0	0	I
	19	15	0	100	0	0	I
	1	88	75	0	0	25	II
	6	162	72	0	0	28	II
	7	36	69	0	3	28	II
	9	104	70	0	0	30	II
	10	44	61	0	0	39	II
	14	97	65	0	0	35	II
	15	103	69	0	0	31	II
	16	54	72	0	0	28	II
	3	90	100	0	0	0	III
	4	50	100	0	0	0	III
	8	167	100	0	0	0	III
	12	118	100	0	0	0	III
	13	204	100	0	0	0	III
	17	70	100	0	0	0	III
	5	109	99	0	0	1	III or II

Independent Leu$^+$ transformants of strain DBY931 with the indicated plasmid were picked up and Ura$^+$ derivatives were isolated and subcloned, selecting only Ura$^+$. Individual colonies resuspended were plated on YEP-glucose; when colonies had appeared they were replica plated to minimal plates lacking leucine or uracil to score the phenotypes. Assignment to classes was done as described in the text.

Ura$^-$Leu$^+$ (the original phenotype of the transformants) segregants were very rarely observed. This indicates that virtually all of the plasmids in the population carry the *URA3*$^+$ gene. Physical analysis (not shown) of class II recombinants showed the presence of multiple copy plasmids similar in every way to

the original transforming plasmids pRB30 or pRB29. In addition, plasmids isolated from class II recombinants, when introduced back into strain DBY931, yield transformants that are both Ura$^+$ and Leu$^+$, unlike the Ura$^-$Leu$^+$ transformants obtained with the parent plasmids. Class II recombinants are easily accounted for by the same explanation used for class I: double crossovers or gene conversion events replaced the *ura3-3* allele with wild-type information from the chromosome. Simple reversion of the *ura3-3* would also explain individuals in class II, but the frequency data (Tables 2 and 3) strongly suggest that most such Ura$^+$ derivatives are indeed recombinants.

Initially, it seemed surprising that all copies of the plasmids in class II recombinants were *URA3*$^+$, since the recombination event(s) almost certainly involved only one of the many plasmids in a cell. Possibly only one of the many is in the nucleus and, thus, able to recombine and to replicate. However, such an explanation is probably not necessary, since in these experiments 75–100 generations and several subclonings intervened between the time of selection of the Ura$^+$ derivatives and the final screening to determine the class of recombinant. This number of generations would suffice for the recombinant plasmid to be segregated from its parental sisters if one assumes independent segregation.

Class III recombinants showed complete correlation of the Ura$^+$ and Leu$^+$ phenotypes: both are stably maintained. This suggests that the entire plasmid became stably integrated into the chromosome, or, less likely, that more than one recombination event involving both the *LEU2* and the *URA3* loci occurred. Physical analysis showed that, indeed, the entire plasmid is integrated into the yeast chromosome at either the *LEU2* or the *URA3* locus (FALCO *et al.* 1982). Class III recombinants apparently arise from a single reciprocal crossover between plasmid and chromosome.

Table 4 shows that the three classes of recombinants are about equally frequent, suggesting that class III recombinants are not strongly selected against. Since class III events involve integration of 2μ DNA into yeast chromosomes, and in the case of recombinants descended from pRB30, the integration of the 2μ circle replication origin as well, such integration must not be lethal.

Fine structure mapping using plasmids: The data given before suggest that the frequency of plasmid-chromosome recombination is high, especially over short distances. This encouraged us to attempt high-resolution mapping of chromosomal mutations using a series of well-characterized deletions of the *URA3* gene which were constructed on YEp plasmids (ROSE, CASADABAN and BOTSTEIN 1981; ROSE 1982; ROSE, GRISAFI and BOTSTEIN 1983). The *URA3* gene has been studied in some detail, and the complete nucleotide sequence has been determined (ROSE 1982; ROSE, GRISAFI and BOTSTEIN 1983) as well as the location in the sequence of many deletion mutations and several point mutations (including *ura3-3*).

The deletion-bearing plasmids were constructed as *URA3-lacZ* fusions in the YEp plasmid pRB45 (ROSE, CASADABAN and BOTSTEIN 1981) which is virtually identical with pRB27, having the same (*ORI*$^-$) fragment of the 2μ circle DNA, the same fragment carrying *LEU2* and virtually the same fragment carrying

URA3. Thus, the only substantial difference between pRB30 and the deletion plasmids used is that the *lacZ* gene replaces a variable amount of one end of the *URA3* gene. The deletions enter the gene from the end encoding the carboxy end of the protein. The chromosomal mutations used were *ura3-3*, *ura3-18*, *ura3-50* and *ura3-52*. The latter two mutations are very stable: *ura3-50* is probably a small deletion, and *ura3-52* is a Tyl insertion mutant (M. ROSE and F. WINSTON, personal communication).

The deletion plasmids were introduced (selecting Leu^+) into haploid strains of yeast bearing one of the aforementioned *ura3* alleles. Transformants were subcloned and grown up in the absence of leucine, thus maintaining selection for the plasmid. Cells in each culture were then replated on medium lacking uracil to determine the number of Ura^+ recombinants. The results are given in Table 5, which also shows the extent (in terms of the nucleotide sequence) of the *ura3* deletion on the plasmid. It is clear that recombinants are observed in some cases (six- to 15-fold over the background frequency) and not in others, and that the appearance of recombinants allows the deduction of a map position relative to the deletion endpoints that is completely consistent with the prior information, which indicated an allele order 5′-*ura3-3*, *ura3-52*, *ura3-50*, *ura3-18*, -3′ (LOSSON and LACROUTE 1979).

In crosses between chromosomal point mutations and deletions on plasmids, the recombination frequency is affected by the distance of the point mutation from the deletion endpoint. The data in Table 5 show that the recombination between *ura3-3* and the deletions ending nearby is two- to threefold less than the recombination observed using deletions only about 150 basepairs (bp) farther away.

The position of the *ura3-3* mutation has been determined by direct DNA sequencing (ROSE 1982): it is an A to T transversion mutation at bp 245 that changes an AAG (lysine) codon to TAG (amber). It recombines (about fivefold over background) with plasmids pRB73 and pRB74, which have their deletion endpoints at bp 270 and 279, respectively. Thus, the resolution of simple spontaneous plasmid-chromosome recombination is clearly adequate to resolve mutations 25 bp apart.

DISCUSSION

The experiments described demonstrate that genetic interactions occur between chromosomal DNA and homologous DNA segments carried on "episomal" plasmids that replicate autonomously in yeast, because they carry a fragment of the 2μ circle DNA. In some cases, the 2μ circle DNA in the plasmids included its origin of replication; in other cases the plasmids were lacking the origin but were still able to replicate autonomously by recombining with resident 2μ circles of the host cell as described previously (BROACH and HICKS 1980). Genetic recombination was observed at the same frequency with both types of plasmids, indicating that all such plasmids have ready access to (and most likely reside entirely within) the nucleus.

Recombination between plasmids and chromosomes occurs in haploid and diploid cells; thus, there are no cell-type specific functions required. In several

TABLE 5

Fine structure mapping using deletions on plasmids

Strain	Chromosomal *ura3* allele	Plasmid	Deletion endpoint[a]	Frequency of Ura^+ ($\times 10^{-7}$)
FY188	*ura3-3*	None		2.0
FY188	*ura3-3*	pRB71	234	1.0
FY188	*ura3-3*	pRB73	270	14.0
FY188	*ura3-3*	pRB74	279	11.0
FY188	*ura3-3*	pRB78	440	29.0
DBY745	*ura3-52*	None		<0.2
DBY745	*ura3-52*	pRB74	279	<0.2
DBY745	*ura3-52*	pRB78	440	8.7
DBY689	*ura3-50*	None		<0.1
DBY689	*ura3-50*	pRB80	688	<0.1
DBY689	*ura3-50*	pRB81	776	<0.1
DBY689	*ura3-50*	pRB82	880	1.3
FY189	*ura3-18*	None		<1.0
FY189	*ura3-18*	pRB80	688	<1.0
FY189	*ura3-18*	pRB81	776	<1.0
FY189	*ura3-18*	pRB82	880	<1.0

[a] The endpoints of the deletions in the *URA3* gene were determined by DNA sequencing (ROSE 1982) and are given as the number of base pairs (bp) from the end of the 1170 bp *Hind*III fragment that carries the *URA3* gene. The *URA3* coding sequence begins (amino terminal) at bp 227 and ends at bp 1030. The deletions remove the carboxy end of *URA3* to the position listed.

different pairwise combinations of alleles, recombination frequencies observed in plasmid-chromosome crosses were comparable to the frequencies found in standard mitotic crosses using the same allele pairs. These results suggest that plasmid-chromosome recombination is dependent upon distance between the recombining mutations. The frequency of recombination in plasmid-chromosome crosses is unaffected by which of a pair of mutations is plasmid borne: reciprocal crosses gave the same recombination frequency.

Although the mutation carried on the plasmid is present in many (ten to 20) copies per cell (see FALCO *et al.* 1982), the recombination frequency is not increased correspondingly over that observed in a heterozygous diploid carrying a single copy of each mutant allele. This observation could be taken to indicate that only one (or a few) of the plasmid copies are available in the nucleus. We prefer the explanation that these plasmids are limited in their ability to recombine by the relatively short (5.5 kb) available homology to the chromosome. A small (twofold) increase in recombination was observed in plasmid-chromosome crosses in diploids, suggesting the possibility that the copy number of the chromosomal allele is rate limiting; however, other explanations (differences between cell types in plasmid copy number, higher levels of recombination enzymes, or some other enhancement of recombination specific to diploids) have not been excluded.

The recombination frequency is also affected by the length of the DNA homology between plasmid and chromosome (S. C. FALCO, M. ROSE and D. BOTSTEIN, unpublished results). Surprisingly, plasmid-chromosome recombi-

nation is stimulated very little by treatments that strongly enhance mitotic recombination in diploids, such as sunlamp and 260 nm of UV radiation. In diploids as well as haploids, such treatments stimulated plasmid-chromosome recombination only about two- to threefold, compared with the more than tenfold effect seen in control crosses that measured standard mitotic recombination. Similar observations have been made when the same 5.5-kb *Bam*HI *URA3* segment carried by the plasmids used here was integrated on chromosomes other than the normal one at which the gene is located: again sunlamp and UV radiation stimulated recombination only modestly (S. C. FALCO, M. ROSE and D. BOTSTEIN, unpublished observations). These results might be taken to suggest that stimulation by these treatments involves longer stretches of homology than the 5.5 kb held in common in the plasmid-chromosome crosses reported here. However, our results are insufficient to rule out many other possibilities.

Analysis of individual recombinants from plasmid-chromosome crosses revealed three classes of recombinants that appear at about equal frequency. Two classes appear to be the result of recombination events that have left a wild-type allele on the chromosome (class I) or on the plasmid (class II). These types of recombinants could be the result of double crossovers or, more likely, gene conversion events. The third class of recombinants was unexpected and appeared to represent integration of the entire plasmid into the chromosome. Such an integration could be the result of a single reciprocal crossover in the homology shared by the plasmid and the chromosome. Detailed physical and genetic analysis of class III recombinants confirmed directly that such recombinants indeed contain the plasmid integrated into a homologous locus on the chromosomes (FALCO *et al.* 1982).

Previous investigators had not detected 2μ DNA integrated into chromosomes (CAMERON, PHILIPPSON and DAVIS 1977; SIGURDSON, GAARDER and LIVINGSTON 1981), which led to the suggestion that such events might be lethal. This is apparently not the case, since the two kinds of plasmids used here, which between them contain all the 2μ DNA, both can be found integrated into chromosomes. However, integration is a rare event which we only detected by first selecting for genetic recombination between plasmid and chromosome. KIELLAND-BRANDT *et al.* (1980) also reported evidence for chromosomal integration of 2μ circle DNA by homologous recombination. Although chromosomal integration of 2μ DNA is not lethal, it does have striking effects on the genetic stability of the chromosome in diploids (FALCO *et al.* 1982). These novel properties can be used for mapping genes to chromosomes simply and efficiently. This mapping method is the subject of the accompanying paper (FALCO and BOTSTEIN 1983).

Plasmid-chromosome recombination is frequent enough to readily resolve genetically very short distances. We observed a five- to tenfold increase above reversion in a cross between a point mutation and a deletion whose endpoint is only 25 bp away. Thus, it seems likely that the limit of resolution might be of the order of 10 bp or less, making this system comparable to classic prokaryotic fine structure mapping methods. Since recombinant DNA techniques make the formation of deletions on plasmids very easy, YEp plasmid-chromo-

some fine structure recombination mapping can easily be applied generally to yeast genes.

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