Recombination of Plasmids into the *Saccharomyces cerevisiae* Chromosome Is Reduced by Small Amounts of Sequence Heterogeneity

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As a model system for studying the properties of mitotic recombination in the yeast *Saccharomyces cerevisiae*, we have examined recombination between a recombinant plasmid (introduced into the *S. cerevisiae* cell by transformation) and homologous chromosomal loci. The recombinant plasmids used in these experiments contained *S. cerevisiae* rRNA genes. We found that the frequency of integrative recombination is sensitive to small amounts of sequence heterogeneity. In addition, the frequency and specificity of these recombination events are affected by the lengths of the interacting homologous DNA sequences.

Repeated genes are found in all eucaryotes. Although the physical properties of many types of repeated genes have been studied in detail, much less information is available concerning genetic interactions among repeated sequences. Below, we describe a model system that we have used to examine mitotic recombination of the repeated rRNA genes of *Saccharomyces cerevisiae*.

The *S. cerevisiae* rRNA genes are repeated approximately 100 times per haploid genome (19) and are located in a single tandem array on chromosome XII (13). Each rRNA gene contains about 9 kilobases (kb) of DNA and encodes four species of rRNA; the 25S, 18S, 5.8S, and 5S species (2, 17). The 25S, 5.8S, and 18S rRNAs are transcribed as a 35S precursor RNA which is later processed (21). The 5S rRNA is encoded on the opposite DNA strand and is separated from the 35S rRNA species by nontranscribed spacer sequences (9, 22). The positions of the rRNA transcripts within the rRNA gene are shown in Fig. 1.

Most laboratory strains of *S. cerevisiae* have rRNA genes with seven *EcoRI* sites per repeat (2, 3, 16, 17). This type of rDNA is called "form I" and the seven fragments produced by *EcoRI* treatment of this DNA are labeled A through G in order of decreasing size. An *S. cerevisiae* strain containing a variant type of rDNA ("form II") has also been previously described (16).

Form II rRNA genes have only six *EcoRI* sites per repeat. The *EcoRI* site that separates *EcoRI* fragments B and E in form I rDNA is missing in form II genes. Thus, a new fragment (called X') is formed which is equal in size to the sum of the *EcoRI* fragments B and E. In a recent study (M. McMahon, S. Smolik-Utlaut, and T. Petes, unpublished data), a number of other differences have been detected. Most of these changes occurred in the nontranscribed spacer and involved small deletions and insertions (Fig. 1). DNA sequencing of a 424-base-pair *TaqI* fragment surrounding the 5S rRNA gene showed only seven differences between form I and form II: three point mutations and four single base insertions. In heteroduplexes formed between form I and form II genes, no insertion or deletion loops were detectable by electron microscopy (S. Smolik-Utlaut, Ph.D. thesis, University of Chicago, Illinois, 1982).

As described below, we examined the effects of the form I-form II heterogeneity on recombination in *S. cerevisiae*. Hinnen et al. (7) showed that when recombinant plasmids containing *S. cerevisiae* DNA were transformed into *S. cerevisiae* cells, these plasmids recombined with homologous chromosomal sequences. To test the effects of the form I-form II heterogeneity on this type of recombination, we transformed a diploid *S. cerevisiae* strain that had one form I rRNA gene cluster and one form II rRNA gene cluster with a recombinant plasmid that had either form I or form II rDNA. By examining the site of insertion of the transforming DNA, we demonstrated that the form I-form II heterogeneity affects the frequency of integration.

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FIG. 1. Restriction map of the *S. cerevisiae* rRNA gene. The upper portion of the figure depicts the arrangement of the seven *Eco*RI fragments (A through G) of the form I repeat (from data in references 2, 3, 9, 15, and 17). The position of the rRNA transcripts is based on data of Philippsen et al. (17). The bottom portion of the figure shows an expanded restriction map of the sequences containing the nontranscribed spacer; data for both form I and form II rRNA genes are shown. The code for the restriction enzyme cleavage sites is: 1 (*Eco*RI), 2 (*Hae*III), 3 (*Alu*I), 4 (*Sma*I), 5 (*Hind*III), 6 (*Hpa*I), 7 (*Pvu*I), and 8 (*Bgl*II). The complete repeat is 9 kb, and the map is drawn to scale.

MATERIALS AND METHODS

Strains. The diploid *S. cerevisiae* strain (SSU10) used in these studies has the following genotype:

a *ura3-50 leu2-3,2-112 asp5 RDN1-1 lys11 his4 his5*
α *ura3-50 leu2-3,2-112 ASP5⁺ RDN1-2 LYS11⁺ HIS4⁺ HIS5⁺*

The RDN1-1 and RDN1-2 notations indicate form I and form II rDNA, respectively.

The *Escherichia coli* strain QSRO (*leuB* B₁⁻ NaI^r *lac r_k m_k*) was used as a host for plasmid pSS2 and was provided by R. Laux (University of Chicago, Illinois). The *E. coli* strain DB6656 (*trp lac pyrF::μ r_k m_k*⁺) was provided by C. Falco (E. I du Pont de Nemours & Co., Inc., Wilmington, Del.) and was used as a host for plasmids pSS1, pSS41, pSS50, pSS51, pSS52, and pSS53.

Plasmid constructions. The plasmid pSS1 was constructed by using plasmids MB1068 (provided by C. Falco at Du Pont) and pY1rA12 (16). The plasmid MB1068 has a *Hind*III fragment containing the *S. cerevisiae* *URA3⁺* gene inserted into the *Hind*III site of pBR322. The plasmid pY1rA12 has a complete form II rRNA gene inserted into the *Eco*RI site of pMB9 by homopolymer linkers (16). The pSS1 plasmid was constructed by ligating a partial *Hind*III digest of pY1rA12 to a complete digest of MB1068. The ligation mixture was used to transform *E. coli* DB6656 to a Tc^r Ura⁺ phenotype. This procedure selects for insertion of *URA3⁺* into the recombinant plasmid, since the *S. cerevisiae* *URA3⁺* gene complements the bacterial *pyrF* mutation (1).

The plasmid pSS2 was constructed from the plasmids pSS1 and CV9 (provided by J. Hicks, Cold Spring Harbor, New York). The CV9 plasmid has a *Pst*

fragment containing the *S. cerevisiae* *LEU2⁺* gene (18) inserted at the *Pst* site of pBR322. Within the *Pst* fragment, the *LEU2⁺* gene is within a *Sall*-*Xho*I fragment (J. Hicks, personal communication). A *Sall*-

*Xho*I digest of CV9 was ligated to a *Sall* digest of pSS1 to construct pSS2.

The plasmid pSS41 was constructed by a similar procedure to that used for pSS1, except the plasmid pY1(1F-4) was used instead of pY1rA12. The plasmid pY1(1F-4) contains a complete form I rRNA gene inserted into pMB9 by homopolymer linkers (16). Restriction maps of plasmids pSS1, pSS2, and pSS41 are shown in Fig. 2.

The restriction enzyme *Bgl*II cuts both form I and form II rRNA genes into two fragments of about 4.5 kb (Fig. 1). We define the *Bgl*II fragment that contains the *Eco*RI fragments B, E, F, part of A, and part of G as *Bgl*II-IA; the fragment containing the *Eco*RI fragments C, D, part of A, and part of G is *Bgl*II-IB. The homologous portions of the form II rRNA gene will be called *Bgl*II-IA and *Bgl*II-IB. The recombinant plasmids pSS50 through pSS53 were constructed by inserting *Bgl*II fragments of the rDNA repeat into the *Bam*HI site of MB1068. The plasmid pSS50 has the *Bgl*II-IA fragment derived from pY1(1F-4) (14). Plasmid pSS51 has the *Bgl*II-IA fragment derived from pY1rA12 (16). The pSS52 plasmid contains the *Bgl*II-IB fragment derived from the form I plasmid pY1rH10 (16). The plasmid pSS53 has the *Bgl*II-IB fragment from the form II plasmid pY1rC1 (16). The restriction maps of these plasmids are given in Fig. 3.

Genetic analysis. The tetrad dissections were done

FIG. 2. Restriction maps of recombinant plasmids pSS2, pSS41, and pSS1. The thin lines represent rDNA sequences, the thick lines indicate pMB9 sequences, and the zig-zag lines show the polydeoxyadenylic acid-polydeoxythymidylic acid linkers. The *leu2* and *ura3* genes are represented as boxes. A through G represent *EcoRI* fragments as in Fig. 1. The code for restriction sites is: *EcoRI* (□), *BglII* (↓), *HindIII* (●), *Sall* (▽), and *Sall-XhoI* (▼). The sizes of pSS2, pSS41, and pSS1 are 18.9, 16.6, and 16.6 kb, respectively, and the maps are drawn to scale.

by standard procedures (11). The *S. cerevisiae* transformation procedure of Hinnen et al. (7) was used. The amount of DNA used in each *S. cerevisiae* transformation (10^8 cells) varied from 5 to 20 μ g. In the experiments done with plasmids pSS50 through pSS53, 10 μ g of DNA was used per plasmid experiment. Bacterial transformation was done as described by Davis et al. (4).

DNA isolation. Bacterial plasmids were isolated by cesium chloride-ethidium bromide density gradients (4). *S. cerevisiae* DNA was isolated by cesium chloride-Hoechst 33258 dye density gradients (15; D. H. Williamson, personal communication).

In the colony hybridization experiments, restriction fragments from plasmids were used as hybridization probes. Fragments were isolated from agarose gels by the technique of Given and Kieff (6).

Colony hybridization. The colony hybridization procedure (7) was used to determine whether haploid strains contained form I or form II rRNA genes. After lysis on nitrocellulose filters, colonies were hybridized to a 32 P-labeled *EcoRI* restriction fragment called K2. This fragment is derived from single-copy chromosomal DNA sequences at the junction of the form I gene cluster; these sequences are not found in form II *S. cerevisiae* strains (23). Thus, *S. cerevisiae* colonies that hybridize to *EcoRI* K2 have form I

rDNA. Hybridization was detected by autoradiography of the filters after hybridization.

To confirm that the colony hybridization procedure was valid, we isolated DNA from 12 spore cultures (derived from three tetrads of SSU10). The DNA was treated with *EcoRI* and analyzed by agarose gel electrophoresis. For all 12 samples, the ability to hybridize with K2 was correlated with form I rDNA, and failure to hybridize to this probe was correlated with form II.

RESULTS

To examine the effect of sequence heterogeneity on recombination events between plasmid and chromosome, we transformed a diploid strain (SSU10) that was heterozygous for the form I-form II heterogeneity (one chromosome containing 100 form I rRNA genes, the other containing 100 form II rRNA genes) with recombinant plasmids that contained either form I or form II rDNA. In addition to insertions of rDNA, the recombinant plasmids contained selectable *S. cerevisiae* genes (the wild-type *LEU2*⁺ or *URA3*⁺ genes or both). By mapping the position of the selectable genes in the *S. cerevisiae* genome, after transformation, we determined the specificity of the recombination event for each class of plasmid. The details of this analysis are described below.

Evidence for preferential integration of plasmids containing intact form I or form II genes. In the first experiments, transformation was done with plasmids containing the complete form II (plasmid pSS2) or form I (plasmid pSS41) genes.

FIG. 3. Restriction maps of recombinant plasmids pSS50 through pSS53. The same code for restriction sites is used as in Fig. 2. The only representational change is that the thick lines represent pBR322 sequences rather than pMB9 sequences. The plasmids pSS50 through pSS53 are about 10.0 kb, and the maps are drawn to scale.

The *S. cerevisiae* strain used in the transformation (SSU10) was heterozygous for the form I-form II heterogeneity and homozygous for non-revertible mutations at the *leu2* and *ura3* loci. The plasmid pSS2 (Fig. 2a) contained pMB9 plasmid sequences, the wild-type *LEU2*⁺ and *URA3*⁺ genes, as well as an intact form II rRNA gene. This plasmid was used to transform SSU10 to the Leu⁺ Ura⁺ phenotype. Nineteen transformants were analyzed.

The genetic analysis of the transformants had two components. First, the transformants were sporulated and dissected. The segregation of the Leu⁺ and Ura⁺ phenotypes was monitored on omission media. For all 19 transformants (at least five tetrads dissected from each transformant), two spores were Leu⁺ Ura⁺ and two spores were Leu⁻ Ura⁻. This pattern of cosegregation is consistent with the integration of the pSS2 plasmid into a single chromosomal site. The second part of the analysis was to determine whether the pSS2 plasmid was inserted within one of the two copies of chromosome XII (the location of the rDNA) or on a different *S. cerevisiae* chromosome. To find out whether the Leu⁺ Ura⁺ phenotypes segregated with one of the two forms of rDNA, we used the colony hybridization technique developed by Hinnen et al. (7). Colonies derived from spores were lysed on nitrocellulose filters and hybridized to the ³²P-labeled *Eco*RI fragment K2, which was derived from the plasmid pY1rG11. This *Eco*RI fragment contains single-copy *S. cerevisiae* DNA and is located at the centromere-distal junction of form I rDNA with other chromosome XII sequences; it is not found in form II *S. cerevisiae* strains (23). Thus, spore colonies that hybridize to this fragment contain form I rDNA; those that fail to hybridize contain form II.

In 16 of 19 transformants, the Leu⁺ Ura⁺ phenotype cosegregated with form II rDNA. At least five tetrads were examined by colony hybridization for each transformant. In 3 of 19 transformants, the Leu⁺ Ura⁺ phenotype cosegregated with form I rDNA. In all 19 transformants, therefore, the pSS2 plasmid inserted within the rDNA. In addition, the pSS2 plasmid, which contains an intact form II repeat, preferentially inserted within the form II rRNA gene cluster. A chi-square analysis indicates statistically significant deviation from nonspecific insertion at a value of $P < 0.01$ ($\chi^2 = 7.28$).

One explanation of this result is that the form II gene cluster is more competent to integrate exogenous DNA than the form I gene cluster. This possibility was excluded by transforming SSU10 with a plasmid (pSS41) that contained an intact form I repeat in addition to pMB9 sequences and a wild-type *URA3*⁺ gene (Fig. 2b). A total of 44 Ura⁺ transformants were analyzed

by the methods described above. In 33 transformants, the pSS41 plasmid integrated into the form I gene array, and in 11 transformants, the plasmid inserted into the form II array. This result demonstrates that the form II cluster is not more recombination competent than the form I cluster. Furthermore, this experiment shows that a plasmid with an intact form I gene preferentially integrates into the form I cluster ($\chi^2 = 10.02$; $P < 0.01$).

These experiments show that intact form I and form II plasmids integrate preferentially into their homologous arrays. One explanation for this phenomenon is that there is a single site in the form I repeat which directs exchange between form I sequences and a similar site in the form II repeat which directs exchange between form II sequences. If these sites exist, then it should be possible to localize them by transforming SSU10 with plasmids carrying each half of the form I and form II repeat units. The rDNA vectors containing the unique site should recombine specifically with their homologous arrays, whereas the plasmids containing rDNA without these sites should insert randomly into both rDNA clusters. The results of the experiments described below, however, suggest that there are no single sites which can account for the insertional bias observed with pSS2 and pSS41.

Recombinant plasmids containing only half of rRNA do not preferentially integrate into form I or form II rDNA. The restriction enzyme *Bgl*II cleaves both form I and form II genes into two fragments of equal size (16). We constructed four plasmids (pSS50 through pSS53) that contained pBR322 sequences, the wild-type *URA3*⁺ gene, and one of the *Bgl*II rDNA fragments (Fig. 3). In all four plasmids, the orientation of the segment of the rDNA insert relative to the pBR322 sequences was the same. Plasmids pSS50 and pSS53 contain the two halves of form I rDNA; pSS51 and pSS52 contain form II sequences. The plasmids pSS50 and pSS51 contain most of the nontranscribed spacer DNA (Fig. 1 and 3). These spacer sequences contain most of the sequence differences between form I and form II (M. McMahon et al., unpublished data).

These plasmids were used to transform SSU10. As indicated in Table 1, the specificity observed with the complete rRNA genes was reduced or absent in experiments with the half repeats. For the form I plasmids (pSS50 and pSS53), a slight tendency for preferential integration into the form I cluster was observed. This preference is not statistically significant when pSS50 and pSS51 are considered individually (Table 1); when the data for these plasmids are pooled, statistically significant preferential integration is indicated. For the form II plasmids, however, there was not even a slight

TABLE 1. Integrative specificity of plasmids pSS50, pSS51, pSS52, and pSS53

Plasmid	No. of transformants with insertions in:		Chi-square value ^a
	Form I rDNA	Form II rDNA	
pSS50 (<i>Bg</i> III-IA)	25	17	1.17
pSS51 (<i>Bg</i> III-IIA)	21	18	0.10
pSS52 (<i>Bg</i> III-IIB)	21	21	0
pSS53 (<i>Bg</i> III-IB)	25	13	3.18
pSS50 + pSS53	50	30	4.15
pSS51 + pSS52	42	39	0.11

^a Chi-square values were determined assuming that the plasmids inserted without preference into either form I or form II rDNA. A chi-square value greater than 3.84 represents a statistically significant ($P < 0.05$) departure from nonpreferential insertion.

tendency for preferential integration. Although the results obtained with the form I and form II plasmids differ slightly, neither observation is consistent with the hypothesis that a single site within the rRNA gene directs preferential integration.

The data presented in Table 1 are consistent with the possibility that the degree of preference in the integration event is a function of the length of the interacting DNA sequences, since the plasmids with half rRNA genes integrate less preferentially than plasmids with intact rRNA genes. The extent to which the length of the homology influences the integration event must also be affected by base sequence since the form I plasmids pSS50 and pSS53 behave somewhat differently than the form II plasmids pSS51 and pSS52. To explain the preferential integration of the recombinant plasmids containing the complete rRNA genes as well as the lesser degree of preferential integration of the plasmids with half of an rRNA gene, we propose the following sequence of events. The recombinant plasmid enters the cell and initiates heteroduplex formation (8, 10) with either the form I or the form II array. For two sequences with no mismatches, this interaction is stable, and recombination is an efficient process. Mismatched bases, however, can destabilize the form I-form II heteroduplexes and cause them to fall apart before they can be resolved as a reciprocal recombination event. Since the plasmids with half repeats show a reduced tendency to integrate preferentially, we suggest that efficient integration requires a long region (about 9 kb) of perfect homology. If this interpretation is correct, recombinant plasmids with intact rRNA gene repeats should transform more efficiently than those with half repeats. This prediction was tested in the following experiments.

Evidence that plasmids containing complete rRNA genes transform with a higher efficiency than plasmids with half repeats. Since the absolute transformation efficiency in different experiments is variable, we included an internal control in each experiment. To quantitate the transformation efficiency of the half repeats (plasmids pSS50 through pSS53), we added an equivalent amount of pSS2 DNA. Since the plasmids pSS50 through pSS53 contain only one selectable marker (*URA3*⁺), whereas pSS2 has both *LEU2*⁺ and *URA3*⁺, we could monitor the efficiency of transformation of the plasmids containing half repeats relative to pSS2 by determining the relative number of *Ura*⁺ *Leu*⁻ and *Ura*⁺ *Leu*⁺ transformants of the SSU10 strain. The efficiency of transformation of pSS41, containing an intact form I rRNA gene, was also examined relative to pSS2. To determine the transformation efficiency of a plasmid containing an intact form II rRNA gene, we used the plasmid pSS1 which contains the *URA3*⁺ gene and a complete form II repeat (Fig. 2c). The orientation of the rRNA gene repeat in pSS1 is the same as in pSS41.

The summary of these experiments is shown in Table 2. Since equal microgram amounts of pSS2 and the experimental plasmids were added in each experiment, and since pSS2 is larger than the other plasmids, we normalized the number of *Ura*⁺ transformants by multiplying this number by the ratio of the molecular weight of the experimental plasmid divided by the molecular weight of pSS2. Thus, the efficiency of transformation indicated in Table 2 represents the relative efficiency per plasmid molecule compared to pSS2. From the data presented in Table 2, it is clear that the plasmids with the intact rRNA genes transform better than plasmids with half repeats. The plasmids containing complete form I (pSS41) and complete form II (pSS1) repeats transformed equally well. An unexpected finding was that the two *Bg*III halves of the rRNA gene transformed with different efficiencies. For both form I and form II rDNA, the plasmids with the *Bg*III fragment containing the nontranscribed spacer (pSS50 and pSS51) transformed better than the plasmids containing the *Bg*III fragment missing the spacer (plasmids pSS52 and pSS53). Plasmids containing homologous *Bg*III halves transformed with about the same efficiency irrespective of whether they contained form I or form II rDNA. To confirm these conclusions, we did a contingency chi-square analysis for various pairs of transformation experiments (Table 3). This analysis basically confirms our previous conclusions, although in one comparison (pSS50 with pSS41), the difference in transformation efficiencies described was not statistically significant.

TABLE 2. Transformation efficiencies of plasmids containing rDNA

Plasmid	No. of Ura ⁺ transformants	Normalized no. of Ura ⁺ transformants (x) ^a	No. of Leu ⁺ Ura ⁺ transformants (y)	Transformation efficiency (x/y)	Transformation efficiency relative to pSS1 and pSS41 ^b
pSS50	89	47	160	0.29	0.77
pSS51	72	38	187	0.20	0.53
pSS52	14	7	111	0.06	0.16
pSS53	23	12	124	0.10	0.27
pSS1	81	71	190	0.37	0.99
pSS41	54	48	128	0.38	1.01

^a The normalized number of Ura⁺ transformants was calculated by multiplying the number of Ura⁺ transformants by the ratio (molecular weight of experimental plasmid per molecular weight of pSS2). For plasmids pSS50 to pSS53, this ratio was 0.53; for pSS1 and pSS41, the ratio was 0.88.

^b The transformation efficiency for each plasmid was divided by the average transformation efficiency of pSS1 and pSS41 (0.375).

DISCUSSION

We have found that the recombination events between a plasmid containing rDNA and homologous chromosomal rDNA sequences have a number of interesting properties. First, when complete rRNA genes (9 kb of DNA) are present on the plasmid, the integration event is sensitive to small amounts of sequence heterogeneity. This conclusion is based on the observation that plasmids containing complete form I genes integrate preferentially into the form I cluster of a heterozygous form I-form II diploid strain; plasmids with complete form II genes integrate preferentially into the form II cluster. Second, when plasmids with half rRNA genes are transformed into the same form I-form II diploid strain, the tendency for preferential integration is diminished or lost. Third, the efficiency of integration is related to the size of the homologous DNA sequence on the plasmid, since plasmids with a complete repeat transform more efficiently than those with a half repeat. Fourth, the efficiency of integration is also related to base sequence, since the plasmids pSS50 and pSS51 transform threefold more efficiently than the plasmids pSS52 and pSS53.

In current models of recombination, the recombination event is initiated by forming a small heteroduplex region between the interacting DNA molecules (8, 10). The length of the heteroduplex is then expanded by branch migration. Correction of mismatched bases within the heteroduplex can lead to gene conversion. This intermediate (known as the "Holliday" structure) can then be resolved with or without reciprocal recombination for markers flanking the heteroduplex region. The mode of resolution of the Holliday structure is determined by which pair of DNA strands are cleaved to allow the interacting DNA molecules to separate.

Based on this model, we suggest that integration of recombinant plasmids into the chromo-

some occurs by the following sequences of events. First, the plasmids form a heteroduplex structure with the chromosomal sequences. One explanation for the observation that pSS50 and pSS51 plasmids transform more efficiently than pSS52 and pSS53 is that the rDNA sequences in pSS50 and pSS51 are better able to induce heteroduplex formation. Alternatively, it is possible that pSS50 and pSS51 contain a weak replication origin. Such an origin might allow pSS50 and pSS51 to be maintained more stably without insertion than plasmids pSS52 and pSS53 and, therefore, have a better opportunity to integrate. Szostak and Wu (20) observed that plasmids with the same *Bgl*II rDNA fragment as that contained in pSS50 and pSS51 were capable of autonomous replication in some *S. cerevisiae* strains. Although our genetic analysis shows that pSS50 and pSS51 always integrate into the chromosome, it is possible that strain-to-strain variation exists in the ability to maintain extra-chromosomal rRNA genes (V. L. Larionov, personal communication).

After heteroduplex formation between the

TABLE 3. Comparison of transformation efficiencies by contingency chi-square analysis

Comparison	Chi-square value ^a
pSS1 and pSS41.....	0.01
pSS50 and pSS41.....	0.83
pSS53 and pSS41.....	15.64
pSS50 and pSS53.....	10.15
pSS51 and pSS1.....	5.72
pSS52 and pSS1.....	19.77
pSS51 and pSS52.....	7.22
pSS50 and pSS51.....	1.95
pSS52 and pSS53.....	0.40

^a The chi-square was done using the normalized number of Ura⁺ transformants and the number of Leu⁺ Ura⁺ transformants in Table 2. A value greater than 3.84 is statistically significant.

recombinant plasmids and the chromosome, three different types of resolution appear possible: (i) integration by reciprocal recombination, (ii) a nonreciprocal transfer of information between plasmid and chromosome without integration, and (iii) dissociation of the heteroduplex without informational transfer. Of these events, only the first class would be expected to produce a stable transformant containing integrated plasmid sequences. We suggest that the fraction of heteroduplex events resolved by integration is a function of the length and homogeneity of the interacting sequences and that efficient integration requires long (9 kb) regions of perfect sequence homology. Thus, either short regions of homology (such as 4.5-kb half repeats) or incomplete sequence homology (represented by form I-form II interactions) would result in inefficient integration. Although it is surprising that the length difference between a complete repeat (9 kb) and a half repeat (4.5 kb) is significant in determining the specificity and efficiency of integration, the factors controlling heteroduplex formation and stability in vivo and in vitro might be quite different. Esposito and Wagstaff (5) have recently summarized evidence that indicates classical mitotic recombination is associated with long heteroduplexes. We have also observed that mitotic gene conversion events within the rRNA gene cluster frequently involve extremely long (greater than 100 kb) heteroduplexes (S. Smolik-Utlaut, Ph.D. thesis, University of Chicago, Illinois, 1982).

It is not clear to what extent the integration of a plasmid into the chromosome is a good model system for investigating mitotic recombination. Orr-Weaver et al. (12) have shown that integration of linearized plasmids into *S. cerevisiae* chromosomes is affected by some of the same mutations that affect normal mitotic recombination. S. C. Falco, M. Rose, and D. Botstein (personal communication) have shown that the frequency of recombination between plasmid sequences and homologous chromosomal sequences is similar to that observed for classical mitotic exchange. Given these similarities, it is likely that many of the same steps are involved in plasmid-chromosome and classical mitotic recombination. We suggest, therefore, that normal mitotic exchange may also be sensitive to small amounts of sequence heterogeneity.

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