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LA-SUB--93-255

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Transformation-associated recombination between diverged and homologous DNA repeats is induced by strand breaks

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ABSTRACT

Rearrangement and deletion within plasmid DNA is commonly observed during transformation. We have examined the mechanisms of transformation-associated recombination in the yeast *Saccharomyces cerevisiae* using a plasmid system which allowed the effects of physical state and/or extent of homology on recombination to be studied. The plasmid contains homologous or diverged (19%) DNA repeats separated by a genetically detectable color marker. Recombination during transformation for covalently closed circular plasmids was over 100-fold more frequent than during mitotic growth. The frequency of recombination is partly dependent on the method of transformation in that procedures involving lithium acetate or spheroplasting yield higher frequencies than electroporation. When present in the repeats, unique single-strand breaks that are ligatable, as well as double-strand breaks, lead to high levels of recombination between diverged and identical repeats. The transformation-associated recombination between repeat DNA's is under the influence of the *RAD52, RAD1* and the *RNC1* genes.

Key Words: Transformatio[illegible]ombination/DNA divergence/ DNA breaks/rad52, rad[illegible] repeats

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INTRODUCTION

While transformation is routinely used for the introduction of DNA into eukaryotic cells, little is known about the mechanism(s) of uptake, the passage to the nucleus or the consequences of transformation on the DNA itself. Mutations such as deletion are frequently observed when DNA is transformed into primate, rodent or yeast cells (Calos *et al.*, 1983; Ashman and Davidson, 1984; Clancy *et al.*, 1984). High levels of intraplasmid recombination within transforming DNA have been reported for mammalian cells (Waldman and Liskay, 1987; Lin *et al.*, 1990) and yeast (Tschumper and Carbon, 1986). The levels are much higher than anticipated based on spontaneous recombination frequencies during mitotic growth. These observations could explain the observed rearrangements and deletions of human, mammalian and *Escherichia coli* DNA's within yeast artificial chromosomes (YACs)(Matsuoka *et al.*, 1991; Neil *et al.*, 1990; Albertsen *et al.*, 1990 and unpublished) during transformation into yeast. Such alterations are common artifacts in the development of human DNA YAC libraries and may hinder the international effort to map and sequence the human genome. The high levels of recombination could be a reflection of the state of the DNA (i.e., naked, relaxed or nicked) resulting in it being more susceptible to DNA metabolic enzymes.

To understand mechanisms of transformation-associated recombination, we utilized a plasmid transformation system in the yeast *Saccharomyces cerevisiae* wherein the effects of the physical state on recombination could be studied. In addition we examined the importance of homology on the transformation-associated recombination, in part because diverged repeats are common to the chromosomal DNA's of many eukaryotes including humans. Single-strand breaks

(SSBs) that are ligatable within repeats, as well as double-strand breaks (DSBs), lead to high levels (~50%) of recombination between identical repeats during transformation, suggesting "competition" between repair by ligation and by recombination. While DNA divergence is an acknowledged barrier to recombination (discussed in ref. Rayssiguier et al.,1989), we have found that the recombinational repair of SSBs and DSBs (Resnick, 1976; Orr-Weaver *et al.*, 1987; summarized in Thaler and Stahl, 1988; Resnick *et al.*, 1983) largely overcomes such barriers within plasmids. We demonstrate that the *RAD52*, *RAD1* and *RNC1* genes play a role in transformation-associated recombination between the repeat DNA's.

MATERIALS AND METHODS

Strains and media

A series of isogenic strains of *S. cerevisiae* were developed from the RAD+ strain EPY216-2C *(MATa leu2-Δ1 ade2-101 trp1::his6 his7-1 lys2 ura3-52)* that were deleted for the following genes using one-step gene replacement (Rothstein, 1981): *rad1-Δ1* (Thomas and Rothstein, 1989), *rad9-Δ1* (Schiestl *et al.*, 1989), *rad52-Δ1* and *rnc1-Δ1* (Gordenin *et al.*, 1992). The deletions correspond to 70 to 100% of the coding sequences. The deletions were confirmed by Southern blot analysis of restriction digests. Media for the growth of *S. cerevisiae* as well as standard genetic techniques have been described elsewhere (Resnick *et al.*, 1983).

Plasmid isolation and introduction of a site specific single-strand break

The plasmids used in the recombination assay are described in Fig. 1. Four types of plasmids were prepared: i) covalently-closed circular DNA; ii) open circular DNA containing nonspecific nicks; iii) plasmid DNA linearized at a

unique restriction site; and iv) circular DNA specifically nicked at unique site(s). DNA was isolated, and covalently closed and open circular plasmid DNA's were separated using standard centrifugation procedures involving CsCl gradients that contain ethidium bromide. The purified closed circular DNA was linearized by *Bam*HI or nicked by exposing the DNA to *Bam*HI (or *Cla*I) in the presence of ethidium bromide (Shortl and Botstein, 1983). The *Bam*HI site is in one of the two *URA3* genes. Randomly nicked plasmid DNA was obtained by treatment with DNAse I in the presence of ethidium bromide (Greenfield *et al.*, 1975). The nicked DNA was separated from linear and closed-circular DNA by agarose gel electrophoresis, followed by electroelution from the gel, extraction with phenol, and ethanol precipitation. Subsequent examination by gel electrophoresis revealed no linear plasmid in the purified circular DNA fraction.

Yeast transformation

Established methods of transformation involving spheroplasting (Burgers and Percival, 1987), electroporation (Higgins and Strathern, 1991) or lithium acetate treatment (Schiestl and Gietz, 1989) were used; the respective frequencies of transformation were approximately 10^6, 10^5, and 10^4 transformants/ug DNA. Transformants were selected on medium lacking leucine and red *ade2*$^-$ colonies were scored visually. Plasmids were physically analyzed by transferring them to *E. coli*, isolating the plasmid DNA, and digesting with the appropriate restriction enzymes. Putative recombinant plasmids were analyzed similarly.

Measurement of mitotic recombination

Cells were grown in liquid synthetic complete medium lacking leucine to stationary phase. They were washed and plated to synthetic complete medium lacking leucine but containing a small amount of adenine (6 ug/ml) in order to

facilitate the visual detection of the red *ade2*$^-$ colonies. Ura$^-$ to URA$^+$ recombination was determined by plating cells to synthetic complete medium lacking uracil.

RESULTS

Recombination during Mitotic Growth vs. Transformation

In order to assess the frequency and nature of transformation-associated recombination and compare it with recombination during mitotic growth, we constructed plasmids containing direct repeats of the *URA3* gene of *Saccharomyces cerevisiae* or the *URA3* gene of *S. carlsbergensis* flanking an intervening copy of the *ADE2* gene (Fig. 1). Loss of the *ADE2* gene function by either mutation or recombination results in Leu$^+$ cells giving rise to red rather than white colonies on appropriate medium. Recombination within the *URA3* repeats could be determined since one or both copies were mutated (discussed below). By using a *URA3* gene from each yeast, the importance of homology to repeat recombination could be examined as the two *URA3* genes are approximately 19% diverged (the longest stretch of homology is 32 bp over a region of approximately 1.0 kb) (C. Gjermansen, Thesis, University of Copenhagen, 1991, and in preparation).

The average frequency of red colonies arising from mitotically growing cells containing a plasmid with identical *URA3* repeats (*S. cerevisiae* or *S. carlsbergensis*) was ~5×10^{-4} (Table 1). A similar frequency was observed for intragenic recombination within the plasmid leading to Ura$^+$. For the plasmid containing homoeologous repeats, the frequency was less than 10^{-5},

demonstrating that divergence can inhibit spontaneous mitotic recombination, as had previously been shown for meiotic recombination between homoeologous chromosomes (Nilsson-Tillgren *et al.*, 1981; Nilsson-Tillgren *et al.*, 1986). The reduced appearance of intragenic recombinants (Ura^{+}) for the diverged vs. identical repeats correlates with the reduced frequency of red colonies (i.e., cells have lost the *ADE2* gene)(Table 1).

These results for recombination between repeats within established plasmids in mitotically growing cells contrast with those for recombination in covalently closed circular plasmids undergoing transformation (using spheroplast or lithium acetate treated cells). The frequency of red colonies among transformants varied from 1 - 4% for the plasmids with identical repeats and 0.1 - 0.4% for plasmids with homoeologous repeats. The high level of transformation-associated recombination did not reflect a general change in capability for recombination in cells undergoing transformation (as was shown for electroporation (Higgins and Strathern, 1991). When cells were exposed to conditions used during transformation or transformed with non homologous DNA's, the frequency of red colonies did not increase for established plasmids in mitotic cells (data not shown). The apparent recombination in transforming plasmids was not due to a mixed population of plasmids arising from *E. coli* since physical analysis indicated that the plasmids used for transformation were homogeneous in size and structure (data not shown). Furthermore, the level of recombination was reduced over 20-fold in a recombination-defective *rad52* deletion mutant (discussed below).

The level of transformation-associated recombination is dependent in part on the method of transformation (Table 2). Following electroporation the frequency of recombination between the homologous repeat genes was approximately 10-fold lower than with procedures involving spheroplasting or

treatment with lithium acetate. Possibly the time required for DNA entry into the nucleus is less with electroporation than with the other methods thereby rendering plasmids less susceptible to DNA metabolic enzymes prior to plasmid establishment.

Transformation-associated Recombination Induced by SSBs and DSBs

The transformation-associated recombination was dependent on the state of the DNA at the time of transformation. The above experiments utilized DNA isolated from *E. coli* which was mostly supercoiled (approximately 90%). Breaks in the plasmid DNA resulted in increased recombination. Random nicking of plasmids by pancreatic deoxyribonuclease in the presence of ethidium resulted in a nearly 5-fold higher level of intraplasmid recombination as compared to supercoiled DNA although the two DNA's were comparable in transformation efficiency (Table 1). Similar results were obtained after exposing plasmid DNA to ionizing radiation (from a ^{137}Cs source). A dose of 4 krad corresponding to an average of approximately one single-strand nick per plasmid (no double-strand breaks were detectable at this dose), resulted in a 3-fold increase in transformation-associated plasmid recombination.

Based on the following results, we propose 1) that SSBs are the primary source of the stimulation of recombination during transformation and 2) that the stimulatory effect requires that the SSBs are located within the repeats. Supercoiled DNA was isolated and [illegible] was produced (Shortl and Botstein, 1983) at the *Bam*HI site within the *URA3* gene or at a *Cla*I site 5 bp away in the polylinker or over 1 kb from *URA3* (Fig. 1; for *Cla*I treatment, there was a mixture of plasmids containing a single nick at one or both of the *Cla*I sites). As shown in Table 3, approximately half of the transformants yielded red colonies

when the nick was in one of the two homologous repeats. Similar results were obtained using a spheroplast method of transformation (data not shown). For the plasmid containing diverged repeats the frequency of red colonies was one-third the value for homologous repeats. These results contrast with those obtained using plasmid DNA's that have been nicked outside homologous repeats in that approximately 5% of the colonies were recombinant which, as mentioned above, is comparable to the frequency obtained with CsCl purified plasmids from *E. coli.*

That the red colonies were due to recombination was shown by transferring the putative recombinant plasmids into an *E. coli* recA strain and subsequently analyzing the plasmids by restriction cleavage. Among 20 red colonies that arose after transformation with closed circular DNA and 10 isolated after transformation with DNA nicked within one of the repeats, 18 and 10, respectively, exhibited restriction patterns expected for recombination between the two homoeologous *URA3* genes (15 restriction sites within the repeats were analyzed)(in preparation). Among the 28 recombinant plasmids, 26 had a single "breakpoint" and the remaining plasmids had two breakpoints within the repeats. The other 2 plasmids that retained both copies of the *URA3* genes were likely due to mutation of the *ADE2* gene consistent with the report that transformation can be mutagenic (Clancy *et al.*, 1984). The level of mutation was comparable to that observed for transformation with a similar plasmid containing only a single copy of *URA3* ($<10^{-4}$ red colonies)(data not shown). Following transformation of yeast with closed circular DNA containing homologous repeats, plasmids were isolated from red colonies and analyzed; among 50 examined all were recombinant (i.e., resulting from deletions presumably by crossovers within the repeats). We conclude that during transformation a SSB is highly efficient at

stimulating recombination between DNA repeats--either diverged or homologous--and that the SSB within a repeat participates directly in the recombination.

Since DSBs are known to induce recombination, we compared the effects of SSBs with DSBs on both transformation-associated recombination and transformation. For homologous direct repeats the levels of recombination were comparable while for diverged repeats, DSB's (sticky ends) were nearly four-fold more efficient at inducing recombination (Table 3). The transformation efficiency was compared between closed circular plasmids and plasmids that had a SSB or a DSB inside or outside one of the repeats (Table 3). The transformation frequencies were comparable for supercoiled plasmids and plasmids with a SSB. Similar frequencies were found for plasmids with a SSB or a DSB external to the repeat (data not shown). However, a DSB in one of the homologous or diverged repeats led to a 50-fold or 10-fold increase in transformation frequency, respectively. The difference in transformation efficiency between plasmids containing a SSB vs. a DSB suggests that the SSB-induced recombination is not due to a SSB being processed to a DSB. This is also supported by the observed differences in transformation-associated recombination between a wild type and a *rad1* mutant (see below). However, the enhanced transformation efficiency might also be due to a combination of increased penetration efficiency of linear DNA's (as compared to circular DNA's) during transformation and recombinational repair of DSBs within repeat DNA's.

Genetic Control of Transformation-Associated Recombination.

We examined the genetic control of transformation-associated recombination induced by SSBs or DSBs using isogenic strains containing

complete deletions of either the *RAD52* gene or the *RAD1* gene. The former is central to many aspects of recombination and is required for DSB recombinational repair (summarized in Thaler and Stahl, 1988; Resnick *et al.*, 1993) and the latter appears to be involved in recombination between repeat DNA's as well as excision repair of DNA lesions (Thomas and Rothstein, 1989; Schiestl and Prakash, 1988 and references therein). Since recombination may contribute to plasmid survival, the results are expressed as "yield," the frequency of transformed colonies with recombined plasmids multiplied by the relative transformation efficiency (x 100). For the linearized plasmids, recombinants among transformants is high while the level of transformation is nearly 500 times lower in the *rad52* as compared to the wild type strain resulting in a low yield of recombinants. As shown in Table 3, the *RAD52* gene product is required for high yields of both SSB and DSB induced recombinants. The *RAD1* gene appears involved only with SSB initiated recombination. We also examined the effects of mutations in *RAD9* and *RNC1* genes. The *RAD9* gene which plays a role in cell signaling in response to chromosomal (Hartwell and Weinert, 1989) and plasmid DNA damage (Bennett *et al.*, 1993), does not influence the transformation-associated recombination. The *RNC1* gene codes for a novel G-protein appears to be involved in recombination (Chow *et al.*, 1992). Transformation associated recombination was reduced nearly 2 to 3 fold in a *rnc1* deletion mutant.

DISCUSSION

Transforming DNA is genetically unstable. During yeast transformation high levels of mutation (Clancy *et al.*, 1984), intraplasmid recombination (Tschumper and Carbon, 1986), and *inter*molecular recombination between diverged and

homologous plasmid DNAs (Ma *et al.*, 1987; Pompon and Nicolas, 1989; Muhlrad *et al.*, 1992) have been observed. However, there have been only limited systematic studies of the source(s) of transforming DNA instability. We anticipated that the state of the DNA (i.e., relaxed, nicked, linear or lacking proteins) would influence the capability for recombination, particularly between diverged DNAs. For example, while it was shown in yeast that DSBs can stimulate transformation associated recombination between diverged plasmid DNAs (Mezard *et al.*, 1992), there may be other sources of instability. The plasmid system we used provided the opportunity to identify sources of genetic change during transformation and to understand limitations on recombination between diverged vs homologous DNAs during transformation or in established DNAs within mitotically growing cells.

There is a considerable difference between plasmid recombination in mitotically growing cells vs during transformation. First of all recombination is much higher during transformation. The transformation-associated recombination is not due to a general increase in cellular recombination induced indirectly by transformation. Secondly, there is over a 100-fold difference in recombination between diverged DNAs in established plasmids as compared to a difference that can be as little as 3 to 4-fold during transformation. Most of the transformation-associated recombination between repeat DNA's during transformation can be attributed to single-strand nicks within the repeats. Presumably the nicks are processed during the transport of the DNA to the nucleus or subsequently during plasmid establishment to give rise to recombinants. There appears to be no contribution by nicks external to the repeats.

The results with circular plasmids that are closed vs plasmids that are nicked external to the repeats contrast with those of Tschumper and Carbon (1986). They observed that the excision of a Ty transposon (via the small δ repeats) during transformation is higher in supercoiled DNA as compared to relaxed plasmid DNA (cut external to the repeats and religated). In our experiments plasmids that are supercoiled always yield comparable or lower frequencices of recombinant products as compared to plasmids nicked external to the repeats. The reasons for the differences remain to be determined. Possibly there is specific recognition of δ sequences within the cell that could affect the potential for recombinational interactions.

We have found that the recombinational repair of SSBs as well as DSBs in yeast (Resnick, 1976; Orr-Weaver et al., 1987; Pompon and Nicolas, 1989; summarized in Thaler and Stahl, 1988 and Resnick *et al.*, 1993) largely overcomes possible barriers due to DNA divergence within plasmids (this report), between plasmids (Pompon and Nicolas, 1989; Ma *et al.*, 1987; Mezard *et al.*, 1992) and in chromosomes (Resnick *et al.*, 1992). While, recombination is generally considered to be influenced by levels of homology and systems that monitor mismatches have been proposed to influence the potential for recombination between diverged DNAs (discussed in Rayssiguier *et al.*, 1989), there have been no systematic studies addressing the potential for transformation associated recombination as a function of homology or state of the DNA. The present results establish that the amount of homology required for efficient repair of SSBs and DSBs is low, suggesting that the concept of a "minimal efficient processing segment" (MEPS) (Shen and Huang, 1986, 1989) may not apply to DNA containing either type of break as the initiating lesion in transforming

DNA. In the region of the *Bam*HI break, the longest stretch of precise homology to the left and the right of the break is only 8 and 5 bp, respectively.

Recently, Mezard *et al.* (1992) demonstrated that recombinant molecules could be recovered from highly diverged (27%) linear molecules following transformation into yeast. They demonstrated recombination within short identical sequences (2 to 12 bp) and suggested common mechanisms for homologous and homoeologous plasmid recombination in the transforming DNA(s). Our observation that recombination between homologous and diverged repeats is similarly decreased in *rad1* and *rad52* mutants supports the idea of common mechanisms. The increased potential for recombination of transforming DNA could explain integration of nonhomologous DNA (Schiestl and Petes, 1991) and incorporation of single-stranded mismatched oligonucleotides (Moerchell *et al.*, 1988) into chromosomal DNA in yeast.

The mechanisms for processing of nicks to yield recombinant plasmids remains to be elucidated although the results presented here indicate that the SSBs are not processed to DSBs. If SSBs are not converted to DSBs, the observation that SSBs are also efficient at inducing recombination appears at odds with results from experiments involving ionizing radiation. Ionizing radiation is much more efficient at inducing SSBs than DSBs (Bradley and Kohn, 1979 and references therein), and yet radiation-induced recombination and survival correlates with DSBs (Resnick, 1976). Possibly there are different categories of SSBs; those due to ionizing radiation often do not have simple 3'OH - 5' PO4 ends (Henner *et al.*, 1983).

While the SSBs in the present experiments should be subject to DNA ligation or a template directed repair, they primarily lead to interactions with

related DNA's when the opportunity is present (a SSB immediately adjacent to a repeat is over 10-fold less efficient at inducing recombination)(data not shown). It is interesting to note that SSBs have been correlated with recombination during meiosis in a *rad52* mutant (Resnick *et al.*, 1984; Resnick *et al.*, 1986), leading to unstable recombinants. Possibly SSBs are highly recombinogenic under specific conditions, such as when present in meiotic chromosomes or in transforming DNA entering wild type cells.

We have also examined the genetic control of transformation-associated recombination. The *RAD1* and *RNC1* genes play a small but significant role. Similar to previous observations (Tschumper and Carbon, 1986), much of the recombination in supercoiled plasmids is mediated by the *RAD52* gene. Furthermore, we have shown that for plasmids containing a nick in one of the repeats, the yield of recombinants is nearly 400-fold lower than in RAD^+.

It is noteworthy that transformation-associated recombination might be an important confounding factor in the cloning of human DNA's into yeast as artificial chromosomes. This recombination may be responsible for many of the artifacts observed in the development of YAC libraries containing mammalian DNA including those used in the mapping and sequencing of the human genome (Neil *et al.*, 1990; Albertsen *et al.*, 1990; Schlessinger, 1990; Green *et al.*, 1991) or for model human DNA containing YACs (in preparation). Our results with repeat DNA's that are diverged to levels observed for ALU and LINE sequences (i.e., up to 15-20%) suggest that SSBs could be a major source of deletions and presumably rearrangements. Such SSBs might arise during the manipulation, including transformation, of large DNA molecules. They also might be present within chromosomal DNA as a result of normal replication or transcription. Based on our results there is the potential for genetically reducing

transformation-associated recombination in yeast. Understanding the reasons for high recombinogenicity of transforming vs. established DNA may also help to elucidate the mechanisms and control of chromosomal recombination in mitotic and meiotic cells.

ACKNOWLEDGEMENTS

Support was provided in part by the Department of Energy through an interagency agreement with NIEHS to M. Resnick for human genome studies. We gratefully acknowledge the gift of the *S. carlsbergensis URA3* clone from C. Gjermansen.

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Figure Legends

FIG 1. Plasmids employed in recombination assay. Each plasmid consists of three elements subcloned into the polylinker of pRS315 (see Sikorski and Hieter, 1989): two copies of *URA3*, from either *S. carlsbergensis* (hatched) or *S. cerevisiae* (filled-in) flanking a functional *ADE2* gene (2.5 kb). The homology between the *S. cerevisiae* and *S. carlsbergensis* *URA3* genes lies within the coding regions (C. Gjermansen, Thesis, University of Copenhagen, 1991, and in preparation). The approximate location of the translation start sites are indicated by arrows. The distance between the end of the *S. carlsbergensis* coding region and the beginning of the *S. cerevisiae* coding region is ~3.0 kb, except in plasmid p618, where it is ~2.8 kb. A mutation generated by filling in a *Bam*HI site at position 632 of the *S. carlsbergensis* coding region is indicated by a Δ below the *Bam*HI. A mutation generated by filling in a *Nco*I site at position 205 of the *S. cerevisiae* *URA3* coding region is indicated by a Δ below the *Nco*I. A deletion of the 5' promoter and coding region to the *Sal*I site at nucleotide 183 of the *S. carlsbergensis* coding region is indicated by a gap and brackets. Plasmids p612/1 and p618 are ura^-; plasmids pDU3RAde2 and p612 are URA^+. In plasmid p618, the entire insert (two *URA3* repeats and *ADE2*) is in the opposite orientation relative to the vector than that indicated by the diagram.

TABLE 1 Recombination, mitotic growth- or transformation-associated, between homologous or diverged repeats in centromere plasmids

URA3 repeat[#]	Plasmid recombination frequency			
	In mitotic cells X 10^4 *		During transformation X 10^2 **	
	ade2$^-$	*URA3*$^+$	*ade2*$^-$	
			Closed circular DNA	Open circular DNA
homologous				
carl/carl	2 to 10	8 to 10	1 to 4	10 to 20
cere/cere	2 to 6	--	1 to 4	--
diverged				
p612/1	< 0.1	0.01 to 0.05	--	--
p612	--	--	0.1 to 0.4	--

* Presented are the range of values from five experiments. Cells were grown to stationary phase in fully supplemented defined medium selective for the plasmid (i.e., lacking leucine). The cells were plated after appropriate dilution to either medium containing uracil or lacking uracil to

determine the frequency of URA^{+} recombinants. Red colonies (*ade2*$^{-}$) were scored on medium containing uracil and the ade^{-} phenotype was confirmed.

**Transformants were selected on medium lacking leucine, and the red *ade2*$^{-}$ colonies were scored visually. Two types of plasmids were used: i) covalently-closed circular DNA; ii) open circular DNA containing nonspecific nicks DNA produced by DNAse I treatment (see Materials and Methods). Included in the isolated covalently closed fraction was a small amount of relaxed, open circular DNA (linear DNA was not detected) possibly arising during the isolation. The reported values correspond to the range observed in greater than 10 experiments.

#The plasmids cere/cere and carl/carl correspond to pDU3RAde2 and p618 (FIG.1). The "cere" and "carl" refer to the *URA3* gene originating from either *S. cerevisiae* or *S. carlsbergensis*. The cere/carl plasmid p612/1 was used to monitor mitotic recombination (*URA3*$^{+}$) and p612 was used to measure recombination (red colonies) during transformation because of the availability of the *BamH*I site (FIG. 1). The mutations in the *ura3* coding sequences contained in the carl/carl and the carl/cere plasmids were separated by approximately 450 bp (see Fig. 1).

TABLE 3. Transformation-associated recombination* in centromere plasmids between homologous or diverged repeats induced by SSBs or DSBs internal to the repeats and effects of breaks on transformation and yield of recombinants.

	Homologous**			Diverged**	
	Transforming DNA#			TransformingDNA	
Genotype	Closed circular	nicked	linear	nicked	linear
2a. Recombination					
RAD+	2%	50%	70%	15%	64%
rad52	<0.1%	2%	90%	0.1%	40%
rad1	0.5%	7%	70%	---	---
rad9	2%	---	---	---	---
rnc1	0.7%	20%	---	---	---
2b. Transformation efficiency@					
RAD+	1	1	50	1	10
rad52	0.2	0.02	0.1	---	---
rad1	1	1	50	---	---
rad9	1	---	---	---	---
rnc1	1	---	---	---	---
2c. Yield@					
RAD+	2	50	3500	---	---
rad52	<0.02	0.04	9	---	---
rad1	0.5	7	3500	---	---
rad9	2	---	---	---	---
rnc1	0.7	---	---	---	---

* Recombination corresponds to the production of red colonies and is expressed as percent transformants (i.e., LEU^+) that are red. Plasmids were recovered and analyzed to establish that the *ade2*$^-$ was due to recombination

TABLE 2 Transformation-associated recombination between homologous repeats in centromere plasmid p618 for different procedures of transformation.

Plasmid recombination frequency* during transformation of X 10^2		
Li acetate treated cells	Spheroplasts	Electroporation
1 to 4	1 to 5	0.08 to 0.37

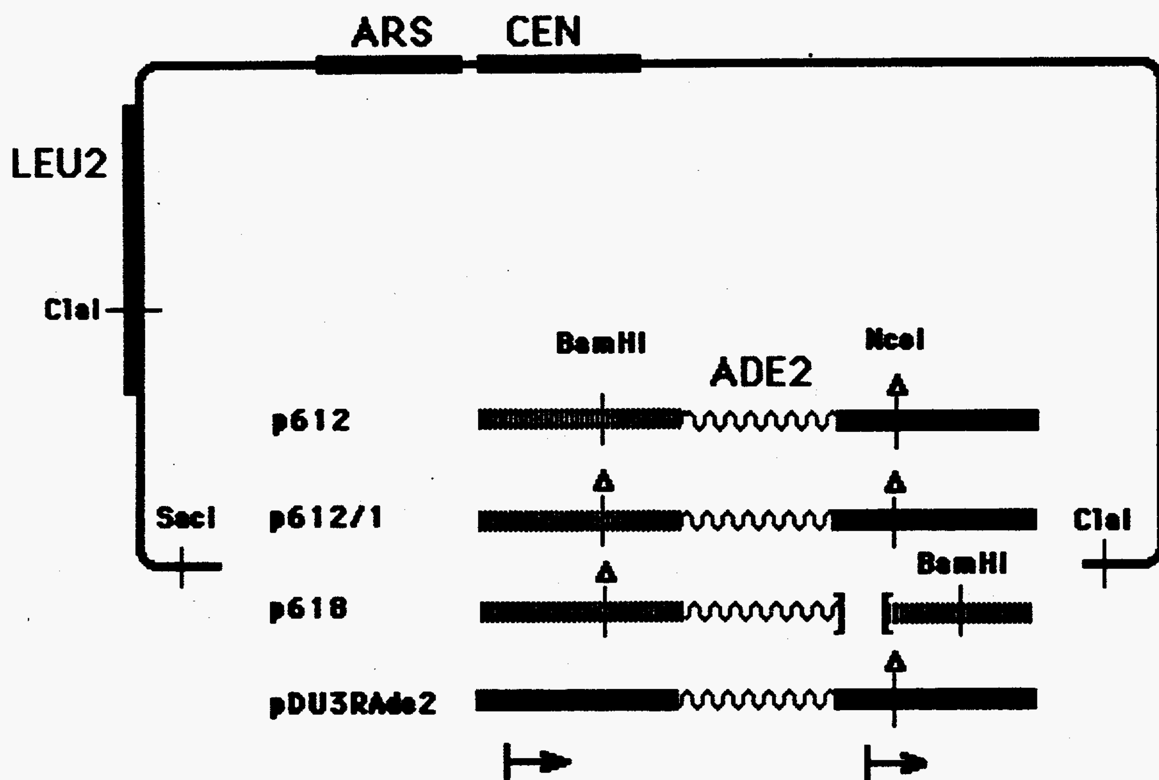
* Presented are the range of values from four experiments. Transformants were selected on medium lacking leucine, and the red *ade2*⁻ colonies were scored visually. Covalently closed fraction of the plasmid p618 was used for transformation. Intraplasmid recombination in red colonies was confirmed by restriction analysis.

(see text). Each result corresponds to the average of greater than 3 experiments.

** Homologous and diverged plasmids refers to plasmids p618 (carl/carl) and p612 (carl/cere), respectively (Fig. 1).

CsCl purified plasmid DNA was either linearized by incubating supercoiled DNA with *Bam*HI or nicked by exposing the DNA to *Bam*HI in the presence of ethidium bromide as described in Materials and Methods. The *Bam*HI site is in one of the two *URA3* genes.

@ The values are relative to transformation in a RAD^+ strain where the frequency is approximately 10^4 transformants/ug for the lithium acetate procedure. The yield is the (frequency of recombinant colonies) x (relative efficiency of transformation) x (100).


ARS
CEN
LEU2
ClaI
BamHI
ADE2
NcoI
p612
p612/1
SacI
ClaI
BamHI
p618
pDU3RAde2