J. Mol. Biol. (1985) **184**, 375–387

Transformation of Yeast with Linearized Plasmid DNA

Formation of Inverted Dimers and Recombinant Plasmid Products

Sam Kunes†, David Botstein and Maurice S. Fox

Department of Biology
Massachusetts Institute of Technology
Cambridge, Mass. 02139, U.S.A.

(Received 27 September 1984, and in revised form 11 March 1985)

The molecular products of DNA double strand break repair were investigated after transformation of yeast (*Saccharomyces cerevisiae*) with linearized plasmid DNA. DNA of an autonomous yeast plasmid cleaved to generate free ends lacking homology with the yeast genome, when used in transformation along with sonicated non-homologous carrier DNA, gave rise to transformants with high frequency. Most of these transformants were found to harbor a head-to-head (inverted) dimer of the linearized plasmid. This outcome of transformation contrasts with that observed when the carrier DNA is not present. Transformants occur at a much reduced frequency and harbor either the parent plasmid or a plasmid with deletion at the site of the cleavage. When the linearized plasmid is introduced along with sonicated carrier DNA and a homologous DNA restriction fragment that spans the site of plasmid cleavage, homologous recombination restores the plasmid to its original circular form. Inverted dimer plasmids are not detected. This relationship between homologous recombination and a novel DNA transaction that yields rearrangement could be important to the cell, as the latter could lead to a loss of gene function and lethality.

1. Introduction

A central feature of putative mechanisms of DNA double strand break repair might be the reactive properties of free DNA ends. Such ends might fuse together randomly or serve as substrates for homologous or illegitimate recombination. Repair could thus restore a broken chromosome unaltered, mutated, recombinant, or rearranged. Recently, with the advent of a technique for the DNA transformation of yeast (Hinnen *et al.*, 1978; Beggs, 1978), plasmid substrates have been utilized to investigate the molecular mechanisms of DNA double strand break repair. When transformation is used to introduce a plasmid DNA, broken or gapped in a region with homology in the yeast genome, repair involving homologous recombination occurs frequently (Hicks *et al.*, 1979; Orr-Weaver *et al.*, 1981). This mode of repair appears similar to the suggested recombinational repair of chromosomal DNA double strand breaks also observed in yeast (Resnick, 1976; Resnick & Martin, 1976). An apparently distinct repair mechanism detected in transformation experiments results in the non-homologous joining of plasmid molecular ends. The re-circularization of a linearized plasmid by apparent ligation has been reported (Orr-Weaver & Szostak, 1983), as has the joining of multiple plasmid restriction fragments in an apparently random manner to form circular plasmid molecules (Suzuki *et al.*, 1983).

We have utilized yeast transformation to introduce plasmid molecules linearized by a cleavage in a region lacking homology with the yeast genome. This would be expected to reveal modes of repair that, like the non-homologous joining of ends, do not require the presence of an intact homologous substrate. Under the conditions that we describe here, which include the presence of sonicated non-homologous carrier DNA during transformation, most transformants are found to harbor a dimer plasmid composed of these linear molecules in the head-to-head orientation, a form not previously reported to be a result of double strand break repair in yeast. Transformants harboring head-to-head (inverted) dimer plasmids are not detected when the transformation is

† Author to whom all correspondence should be addressed.

0022–2836/85/150375–13 $03.00/0

© 1985 Academic Press Inc. (London) Ltd.

performed in the presence of a homologous DNA restriction fragment that includes the site of the plasmid cleavage. Instead, homologous recombination between these two substrates gives rise to a high frequency of transformants harboring the plasmid in its original circular form. Homologous recombination would thus seem to be a mode of repair preferred to that which yields a perfectly or quasi-symmetrical joint at or near the original site of a broken DNA end.

2. Materials and Methods

(a) *Strains and plasmids*

Escherichia coli strain HB101 (Boyer & Roulland-Dussoix, 1969) was used in the construction and preparation of plasmids pSK117, pSK120 and pSK127. Strain HB101 was also the recipient of plasmids recovered from yeast by transformation. Yeast strain DBY1226 (*MATα his4-519 met8-1 leu2-3 leu2-112 ura3-3)* was constructed by standard methods (Sherman *et al.*, 1979).

Plasmids pSK117, pSK120 and pSK127 were constructed by procedures described by Davis *et al.* (1980). Plasmids pCH307 and pCH308 were constructed by Connie Holm (MIT).

(b) *Media*

Yeast was grown in YEPD (complete) or SD (minimal) medium (Sherman *et al.*, 1979). *E. coli* was grown in LB (complete) medium (Miller, 1972) containing, when appropriate, 100 μg ampicillin/ml (Sigma Chemical Co., St Louis, MO). Yeast minimal medium containing the chromogenic indicator X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside; Bachem Fine Chemicals, Torrance, CA) was prepared as described by Rose & Botstein (1983*a*).

(c) *DNA preparation and cleavage*

Plasmid DNA was isolated from *E. coli* by a modification (Rambach & Hogness, 1977) of the method of Clewell & Helinski (1969). Plasmid DNA was further purified by banding once in a CsCl/ethidium bromide density gradient (Radloff *et al.*, 1967). Supercoiled plasmid DNA was stored in TE buffer (10 mM-Tris, pH 8·0, 1 mM-Na_2EDTA) at 4°C or −20°C.

Yeast DNA was isolated by the procedure of C. Holm, D. Wagner, W. Fangman and D. Botstein (unpublished results) from approximately 10^9 cells grown in SD liquid medium (with plasmid selection when appropriate).

Chicken erythrocyte DNA was obtained from Calbiochem–Behring (La Jolla, CA). After dissolution in TE buffer, it was sonicated to an average molecular weight of 5×10^5, phenol/chloroform (1 : 1, v/v, equilibrated to pH 8·0) extracted 3 times, precipitated in ethanol 3 times and stored in TE buffer at 4°C. Phage λ DNA was prepared from phage purified by centrifugation in CsCl (Davis *et al.*, 1980) and then sonicated to yield fragments of an average molecular weight of about 5×10^5. φX174 RFI DNA was obtained from New England Biolabs, Beverly, MA.

Restriction enzymes were used according to the recommendations of the manufacturer (New England Biolabs, Beverly, MA).

(d) *Yeast transformation*

Yeast transformation was performed essentially as described by Hinnen *et al.* (1978) with the exception that STC buffer (1 M-sorbitol, 10 mM-Tris, pH 7·5, 10 mM-$CaCl_2$) was substituted for 1 M-sorbitol in the third wash after treatment with glusalase (Dupont Pharmaceuticals, Wilmington, DE). The final suspension contained spheroplasts at a concentration of about 10^9/ml, of which typically 10% were colony-forming units in regeneration agar with complete supplements. In each experiment, 100 μl of the spheroplast suspension were added to 10 μl of TE buffer containing plasmid DNA and 10 μg of carrier DNA, prepared as described above. Plating dilutions were made in STC buffer. Unless noted otherwise, selection for *Leu2*$^+$ transformants was made on plates containing 1 μg leucine/ml. This was found to maximize the yield of such transformants, presumably by allowing sufficient time for the adequate expression of the plasmid-encoded *LEU2* gene prior to leucine starvation. Under these conditions, when a plasmid contained both the *URA3* and *LEU2* genes, selection for either resulted in similar frequencies of transformants. Plating on selective SD media was followed by incubation at 30°C for 4 to 5 days.

(e) *DNA gel-transfer hybridization*

DNA gel-transfer hybridization analysis of yeast and *E. coli* DNA was performed as described by Page & de la Chapelle (1984).

(f) *Electron microscopy*

Plasmid DNA heteroduplexes were prepared and visualized by electron microscopy by the methods described by Davis *et al.* (1980).

3. Results

(a) *Transformation with a linearized plasmid DNA in the presence and absence of a homologous DNA fragment that spans the plasmid break*

To explore the molecular consequences of DNA double strand break repair that occurs in the absence of an available substrate for homologous recombination, a plasmid linearized by a cleavage in a region lacking homology with the yeast genome was used to transform yeast. We also explored the relationship of this putatively non-homologous repair to homologous recombinational repair by providing a homologous DNA substrate during the transformation. We supposed that a restriction fragment of the plasmid that includes the site of the plasmid cleavage could be a substrate for the recombinational repair of the linearized plasmid. The observation of Hicks *et al.* (1979) that competent yeast spheroplasts are capable of incorporating several plasmid molecules suggests that one could provide this DNA fragment substrate by adding it to spheroplasts along with the linearized plasmid DNA.

A plasmid bearing the cloned yeast *LEU2* and *URA3* genes and capable of autonomous replication in yeast is shown in Figure 1. This plasmid, pCH308, contains a fusion of the *Saccharomyces cerevisiae CYC1* gene to the *E. coli lacZ* gene (Guarente & Ptashne, 1981) that encodes a β-galactosidase activity expressed in yeast. The

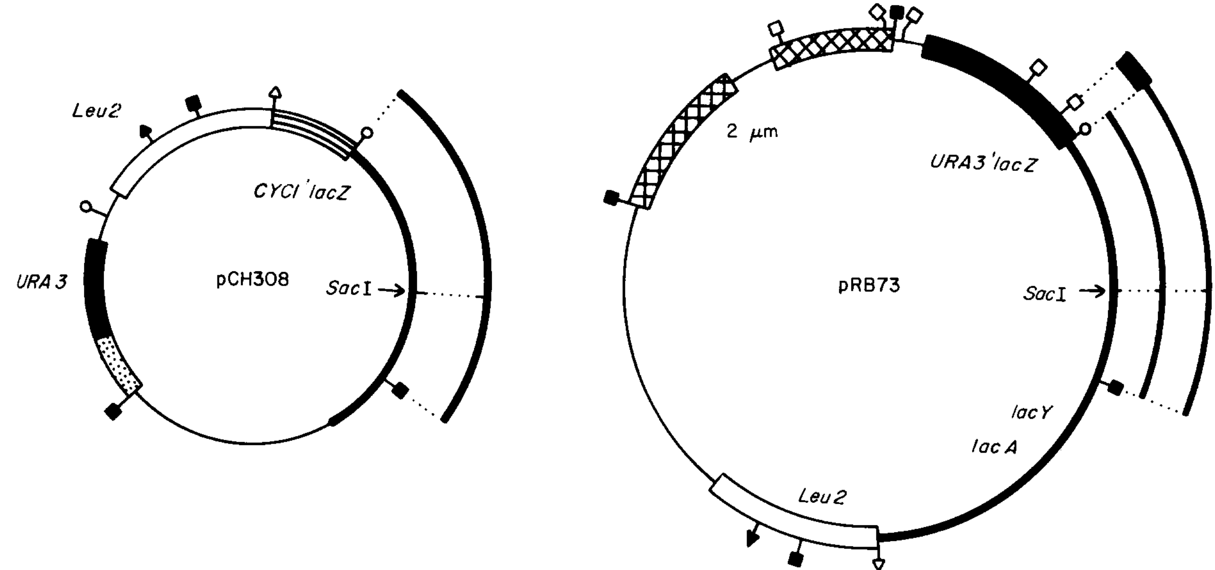


Figure 1. Structure of pCH308, pRB73 and their subcloned fragments. Plasmid pCH308 is a derivative of pBR322 (Bolivar *et al.*, 1977) containing the cloned yeast *URA3* (Bach *et al.*, 1979) and *LEU2* (Ratzkin & Carbon, 1977) genes, the yeast *ARS1* replicator (Struhl *et al.*, 1979), and a fusion of the yeast *CYC1* gene to *E. coli lacZ* (Guarente & Ptashne, 1981). This plasmid encodes β-lactamase and can replicate in *E. coli*. The *Bam*HI-*Eco*RI fragment of the plasmid shown outside the circle was subcloned into a *Bam*HI, *Eco*RI-cleaved pBR322 vector to construct pSK127. This subcloned fragment includes the *Sac*I site in the pCH308 *lacZ* sequence, as is indicated in the Figure. Plasmid pRB73 (Rose & Botstein, 1983*b*) is a derivative of pBR322 containing the yeast *LEU2* gene and a 4·1 kb segment of the endogenous yeast plasmid 2 μm circle that renders it an autonomous plasmid in yeast (as described in the text). It also contains a *URA3'lacZ* gene fusion that encodes a β-galactosidase activity expressed in yeast. Two fragments subcloned from pRB73 are traced outside the plasmid. The *Hin*dIII-*Eco*RI fragment was inserted into the pBR322 *Hin*dIII and *Eco*RI sites, to construct pSK120. The *Bam*HI-*Eco*RI fragment was inserted into the pBR322 *Bam*HI and *Eco*RI sites to construct pSK117. Symbols for restriction enzyme cleavage sites are as follows: (↑) *Kpn*I; (♀) *Bam*HI; (▼) *Eco*RI; (♀) *Hin*dIII; (♤) *Sal*I. pCH308 *Hin*dIII cleavage sites are not shown.

plasmid is cleaved once by restriction nuclease *Sac*I at a site in the *lacZ* sequence. Since a *lacZ* probe does not reveal yeast genomic sequences in gel-transfer hybridization (see Fig. 2 for an example), the molecular ends of *Sac*I-cleaved pCH308 would not be expected to be appropriate substrates for homologous recombination with sequences present in the yeast genome. A restriction fragment of pCH308 that includes the *Sac*I site, and hence might serve as a homologous substrate for the repair of the *Sac*I-cleaved plasmid, was subcloned into the vector pBR322 (Bolivar *et al.*, 1977). This fragment (Fig. 1) extends 1·9 kb† and 1·1 kb to the *Bam*HI and *Eco*RI sites, respectively, on either side of the *Sac*I site.

(i) *Transformation in the absence of a homologous DNA fragment*

Though *Sac*I-cleaved pCH308 would not be expected to be repaired by homologous recombination with a yeast chromosomal locus, transformants could be expected with this DNA since the linear molecules might circularize *via* the non-homologous joining events described by Orr-Weaver & Szostak (1983) and Suzuki *et al.* (1983). Their observations suggest that these events would often restore the *Sac*I site and the *lacZ* coding sequence. Such transformants should express β-galactosidase, which is easily assayed on minimal medium containing the chromogenic substrate X-gal (Guarente & Ptashne, 1981; Rose *et al.*, 1981). As shown in Table 1, *Leu2*$^+$ transformants do result from exposure of the *leu2*$^-$ yeast strain, DBY1226, to sonicated carrier DNA and various amounts of *Sac*I-cleaved pCH308 DNA. These transformants occur at a frequency that is from 2% to 5% that observed with an equivalent amount of closed circular pCH308 DNA. In contrast to the transformants obtained with the closed circular DNA, most of those obtained with the linearized plasmid DNA are phenotypically *lacZ*$^-$ (Table 1). Hence, few of these transformants have restored the *lacZ* coding sequence which had been cleaved by *Sac*I. This outcome of transformation with the linearized plasmid is found to be dependent on the presence of carrier DNA during the transformation (see below). The origin of these transformants is explored below.

(ii) *Transformation in the presence of the homologous* lacZ *DNA fragment*

The outcome is significantly different when the *Bam*HI, *Eco*RI restriction fragment of *lacZ* is

† Abbreviations used: kb, 10^3 bases or base-pairs; IR, inverted repeat(s).

Table 1

Transformation with a linearized plasmid in the presence and absence of a homologous restriction fragment DNA that includes the site of plasmid cleavage

		Plasmid DNA added (ng)							
		pCH308				pRB73			
		100	250	750	2000	50	200	500	3000
CC	*Leu2*$^+$	110	170	380	1000	25	180	500	1000
	lacZ$^+$	115/115	—	—	89/89	52/52	—	55/55	53/53
·*Sac*I	*Leu2*$^+$	2·4	7·2	25	41	1·4	8·4	23	68
	lacZ$^+$	7/87	7/78	10/108	15/110	0/14	—	1/72	1/76
·*Sac*I + *lacZ*	*Leu2*$^+$	66	120	94	94	23	60	76	200
	lacZ$^+$	109/113	89/89	81/82	87/89	84/84	—	91/92	84/84

Transformation of yeast strain DBY 1226 (*ura3-3 leu2-3 leu2-112*) was carried out in the presence of sonicated chicken erythrocyte (carrier) DNA as described in Materials and Methods. The frequencies of *Leu2*$^+$ transformants shown in the Table are normalized to 1000 (closed circular pCH308 at 2000 ng or closed circular pRB73 at 3000 ng), and are about 10^{-2} the actual yield. In those cases in which the subcloned *lacZ* fragment was provided, about 5 μg of the appropriate pBR322 subclone (see Fig. 1), cleaved to liberate the fragment from the vector sequences (*Bam*HI, *Eco*RI-digested pSK127 for pCH308 and *Hin*dIII, *Eco*RI-digested pSK120 for pRB73) were added along with the *Sac*I-cleaved plasmid and carrier DNA. Transformants were tested for their *lacZ* phenotype on yeast minimal medium containing the indicator X-gal in the absence of leucine. Patches that remained white after several days at 30°C were scored as *lacZ*$^-$. *lacZ*$^+$ transformants gave rise to a dark blue patch. Transformants with intermediate phenotypes were rare (less than 1%).

present. Spheroplasts of the *leu2*$^-$ yeast strain, DBY1226, were exposed to DNA mixtures containing a large amount (about 3 μg) of the subcloned fragment, liberated by *Bam*HI and *Eco*RI cleavage from its pBR322 vector, sonicated carrier DNA, and a variable amount of *Sac*I-linearized pCH308 DNA. When the *lacZ* restriction fragment DNA was added along with either 100 ng or 250 ng of the linearized plasmid DNA, the yield of *Leu2*$^+$ transformants was at least an order of magnitude greater than the yield in the absence of the *lacZ* fragment (Table 1). This enhanced yield approaches that observed with closed circular pCH308 DNA. No enhancement is observed if the homologous restriction fragment provided does not include the *Sac*I site or if the *lacZ* DNA fragment has also been cleaved by *Sac*I. With higher concentrations of linearized pCH308 DNA, the yield of transformants is only modestly enhanced by the presence of the *lacZ* restriction fragment DNA (Table 1). When the plasmid DNA concentration dependence is explored in greater detail (data not shown), it is found that below concentrations of about 100 ng, the yield of *Leu2*$^+$ transformants varies in direct proportion with the amount of linearized plasmid DNA added, and is approximately equivalent to the yield with closed circular DNA. The transformation yield, however, consistently saturates at a lower value than with closed circular plasmid DNA, though this plateau is usually higher than in the experiment with pCH308 described in Table 1. The reason for the diminished plateau is not clear. It is possible that the population of cells competent for the uptake of linear DNA molecules is often smaller than that capable of taking up closed circular plasmids. Alternatively, the participation of more than one linearized plasmid molecule in recombination with a homologous DNA fragment could often result in an inviable product.

In contrast to the *Leu2*$^+$ transformants that result with the linearized plasmid alone, nearly all of those formed in the presence of the *lacZ* restriction fragment DNA are phenotypically *lacZ*$^+$ (Table 1). Gel-transfer hybridization analysis (Southern, 1975) of DNA preparations from 12 of these *lacZ*$^+$ transformants detected, in each case, a plasmid that was indistinguishable from the parent plasmid, pCH308. In all 12 cases, the *Sac*I site in the *lacZ* sequence was present and the fragments resulting from *Eco*RI or *Cla*I cleavage were of the appropriate sizes. Hence, consistent with the *lacZ*$^+$ phenotype of these transformants, the site of the *Sac*I-generated break in the *lacZ* coding sequence has been restored. DNA preparations from four of the rare *lacZ*$^-$ *Leu2*$^+$ transformants were also analyzed by gel-transfer hybridization. Each of these was found to contain an apparent plasmid derivative of pCH308 with a deletion encompassing the *Sac*I site. These deletions presumably account for their *lacZ*$^-$ phenotype.

Direct evidence for recombination between a linearized plasmid and a homologous restriction fragment was obtained with genetically marked

substrates. We utilized a mutant derivative of the *URA3'lacZ* fusion plasmid, pRB73 (Rose & Botstein, 1983*b*), shown in Figure 1. The mutant plasmid, pKO111, bears a base change in the putative yeast translation start codon of the fusion protein, which results in a somewhat leaky $lacZ^-$ phenotype (K. Overbye, unpublished results). The mutant allele can thus be conveniently detected; yeast harboring the mutant plasmid yield a pale blue patch on an X-gal indicator plate, whereas the wild-type plasmid confers a dark blue. The site of the base change is in the *URA3* sequence, 49 nucleotides from the *Bam*HI linker at the site of the *URA3* gene fusion with *lacZ*. The wild-type start codon is included in the *Hin*dIII-*Eco*RI fragment of pRB73 subcloned in pSK120 (see Fig. 1), but is not included in the 275 base-pair shorter *Bam*HI-*Eco*RI fragment subcloned in pSK117. Both subcloned fragments include the *Sac*I site in the plasmid *lacZ* sequence.

Transformation of the $leu2^-$ strain DBY1226 was carried out with the *Sac*I-linearized mutant plasmid alone or in combination with either subclone plasmid DNA, the latter cleaved so as to liberate the fragment insert. Both combinations gave a frequency of $Leu2^+$ transformants that was about tenfold greater than with the linearized mutant plasmid DNA alone (Table 2), a similar enhancement to that resulting from the presence of a *lacZ* fragment with *Sac*I-cleaved pCH308. The *lacZ* phenotype of the $Leu2^+$ transformants was then tested on X-gal indicator plates. Those obtained with *Sac*I-linearized pKO111 alone were all white ($lacZ^-$). Most of those obtained with the combination of linearized pKO111 and *Bam*HI, *Eco*RI-cleaved pSK117 (the shorter fragment) showed the original pKO111 *lacZ* mutant phenotype (pale blue), consistent with their having undergone homologous fragment-dependent repair. Approximately 55% of the $Leu2^+$ transformants obtained with pKO111 in combination with *Hin*dIII, *Eco*RI-cleaved pSK120 (the longer fragment) were fully $lacZ^+$ (dark blue), while most of the remainder showed the pale blue pKO111 phenotype. These $lacZ^+$ plasmids have apparently inherited the restriction fragment marker. If the digested pSK120 DNA was provided along with pKO111 in closed circular form, only 1% of the $Leu2^+$ transformants were $lacZ^+$. Therefore, rescue of the restriction fragment marker occurs, for the most part, as a consequence of the repair of the linearized plasmid.

(b) *The structure of the plasmid products of repair that occur in the absence of the homologous* lacZ *fragment*

The $lacZ^-$ $Leu2^+$ transformants, the predominant class obtained with *Sac*I-cleaved pCH308

Table 2

Marker rescue associated with the lacZ *fragment-dependent re-circularization of a linearized plasmid*

	$Leu2^+$	$lacZ^+$/total
cc	4200	0/72
·SacI	200	0/20
·SacI +short	2000	0/145
·SacI +long	1700	71/130

Transformation of strain DBY1226 in the presence of sonicated carrier DNA was carried out as described in Materials and Methods. Approximately 100 ng of plasmid pKO111, a mutant derivative of pRB73 (Fig. 1) described in the text, were added to spheroplasts either linearized by cleavage with *Sac*I or in the closed circular (cc) form. When provided, approximately 5 μg of either pSK117 DNA or pSK120 DNA, cleaved with *Bam*HI and *Eco*RI, or with *Hin*dIII and *Eco*RI, respectively, to liberate the fragment insert (see Fig. 1) were added as a mixture with the linearized mutant plasmid. The pBR73 (wild-type) fragment subcloned in pSK117 (denoted short in the Table) is 275 base-pairs shorter at one end than the pRB73 fragment subcloned in pSK120 (denoted long). The Table gives the total yield of $Leu2^+$ transformants and the fraction of transformants that exhibited the $lacZ^+$ phenotype (dark blue) after scoring on yeast minimal medium containing the indicator X-gal. Transformants exhibiting the leaky $lacZ^-$ phenotype of pKO111 (pale blue) and those exhibiting a completely $lacZ^-$ phenotype (white) account for the remainder of the total. These are not distinguished in the Table since variability in the pKO111 phenotype makes determination of the genotype of the small number of "white" transformants (less than 10%) uncertain.

DNA in the absence of the homologous *lacZ* fragment, were examined for instability of the *Leu2*$^+$ phenotype so as to determine whether it is plasmid-encoded. Twenty of these transformants were colony-purified and then allowed to grow on rich (non-selective) media for approximately 20 generations. For each of these, typically less than 1% of the non-selectively grown population was found to have retained the *Leu2*$^+$ phenotype. This instability is characteristic of a plasmid that, like pCH308, is rendered autonomous by a resident yeast ARS1 segment (Struhl *et al.*, 1979).

The *lacZ*$^-$ *Leu2*$^+$ transformants were further analyzed for the presence of pCH308 plasmid sequences. This was done by gel-transfer hybridization (Southern, 1975) of DNA isolated from the transformants as well as by recovering plasmids from the yeast DNA *via* transformation of *E. coli* to ampicillin resistance, which is encoded by pCH308 (see Fig. 1). Plasmid DNA from the bacterial transformants could then be examined by electron microscopy and restriction analyses.

(i) *Gel-transfer hybridization*

Gel-transfer hybridization of *Eco*RI-digested yeast DNA from 24 *lacZ*$^-$ *Leu2*$^+$ transformants with a probe containing pBR322, *lacZ* and *URA3* sequences (see Fig. 2) gave the following result. One transformant had no detectable plasmid sequences. Two transformants lacked the *Sac*I site-containing 5·0 kb *Eco*RI fragment of pCH308, had the other two pCH308 *Eco*RI fragments (3·5 kb and 4·0 kb) and bore a restriction fragment not present in pCH308 that was somewhat smaller than the *Eco*RI fragment containing the *Sac*I site. These two transformants probably contain pCH308 derivatives with a deletion encompassing the *Sac*I site, a kind of repair product that has been reported (Suzuki *et al.*, 1983). For the remaining 21 transformants, the *Sac*I site-containing *Eco*RI fragment was absent, the two other *Eco*RI fragments of pCH308 were present, and two new restriction fragments of approximately 2 kb and 8 kb were detected. A structure that can account

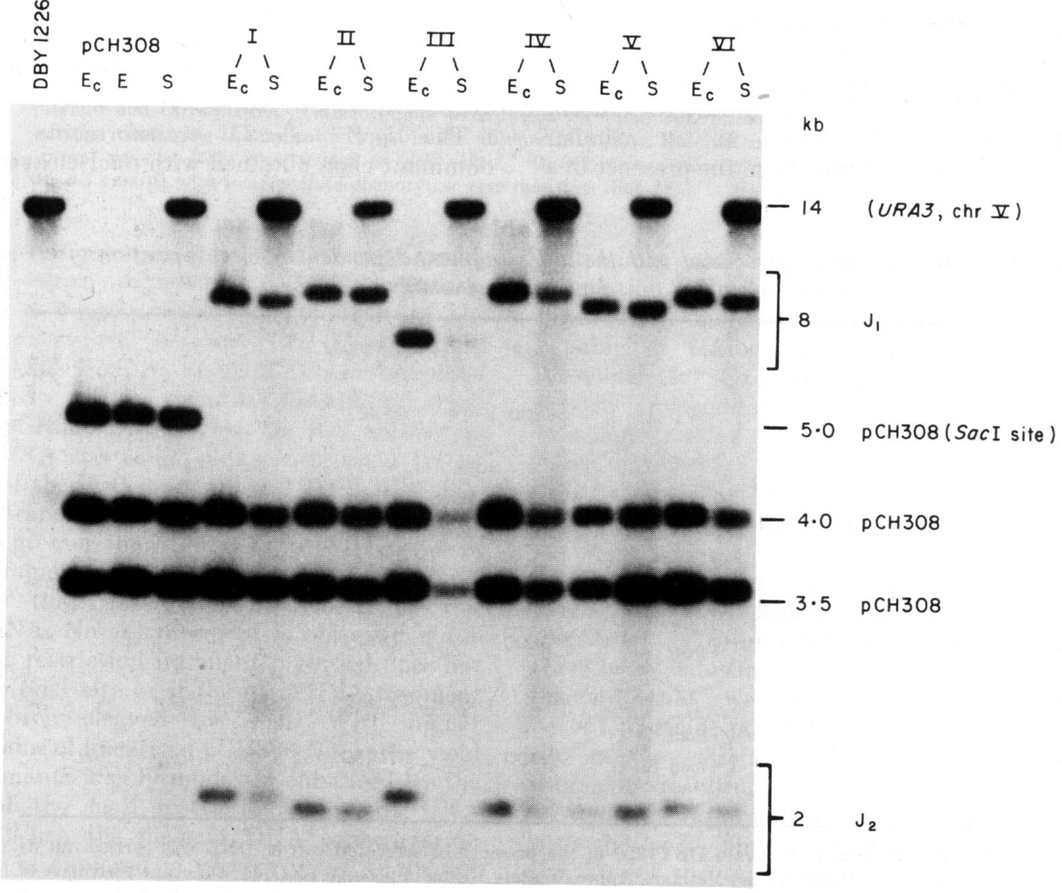


Figure 2. Gel-transfer hybridization of *Eco*RI-cleaved DNA from yeast and *E. coli* transformants. *Eco*RI-cleaved DNA of yeast strain DBY1226, pCH308 (E, purified from *E. coli* strain HB101; E_c, additional purification by banding in a CsCl/ethidium bromide gradient; S, DNA from transformed DBY1226), and of a yeast transformant or its respective *E. coli* transformant harboring plasmids I through VI, transferred to a membrane filter as described by Page & de la Chapelle (1984). Hybridization with a probe containing pBR322, *URA3* and *lacZ* sequences detects the 14 kb *URA3* fragment of yeast (in lanes marked S), and the *Eco*RI fragments of pCH308. Plasmids I through VI are isolated from transformants obtained with *Sac*I-linearized pCH308 DNA, as described in the text. J_1 and J_2 denote the 2 new fragments (absent in pCH308) that appear to be the junction-containing fragments of an inverted dimer (see Fig. 3). Plasmid III appears to be present in yeast at a low copy number, and hence the plasmid bands are faint at the film exposure time shown.

for this pattern is shown in Figure 3. The lengths of the two new fragments are approximately those expected of the head-to-head and tail-to-tail junction-containing fragments of the structure shown, a head-to-head (inverted) dimer of the *Sac*I-linearized plasmid molecule. These end-to-end junctions will hereafter be referred to as "symmetrical" or "palindromic", though, in most cases, whether these plasmid joints contain true or imperfect palindromes is not clear.

The sizes of the junction-containing fragments (denoted J_1 and J_2 in Fig. 3) differ slightly among the 21 inverted dimer plasmids examined, as is evident in Figure 2. These fragments are to a variable extent smaller than would be expected from a conserved joining of the *Sac*I-generated molecular ends. Most of the junction fragments are 100 to 300 base-pairs smaller, though, occasionally a junction fragment is missing more than 1000 base-pairs (see, for example, fragment J_1 of plasmid III in Fig. 2). We suppose that material at the symmetrical joint is to a variable extent deleted. The absence of a *Sac*I site from each of the 42 junction fragments is consistent with this suggestion.

(ii) *Recovery of plasmids in* E. coli

The presence of a perfect DNA palindrome has been shown to render plasmids and λ phage inviable in an *E. coli* host (Leach & Stahl, 1983; Gellert *et al.*, 1979; Collins, 1981). In yeast, however, a plasmid bearing a true DNA palindrome is viable (unpublished result). It is possible then that the putative inverted dimer plasmids detected in the yeast transformants would be unable to propagate in *E. coli*. The yeast DNA preparations containing these plasmids were used in the transformation of *E. coli* strain HB101 ($recA^-$) to ampicillin resistance (Beggs, 1978; Davis *et al.*, 1980). For 11 of the 21 preparations, a small number of transformants to ampicillin resistance were obtained. A low yield of transformants is not unusual for this procedure; with yeast DNA from two transformants harboring pCH308 the yield of HB101 transformants was also low. Thus, while more than half of these yeast transformants clearly harbor plasmids capable of propagation in *E. coli*, the rest may not.

The plasmids detected in the yeast transformants were compared directly with those present in their respective *E. coli* transformants. As shown in Figure 2, the *Eco*RI fragments of six plasmids isolated from yeast are very similar in size to those of the plasmids recovered by transformation of *E. coli*. Two of these six plasmids isolated from *E. coli* were subsequently examined extensively by restriction analysis (Fig. 4). For each, cleavage with

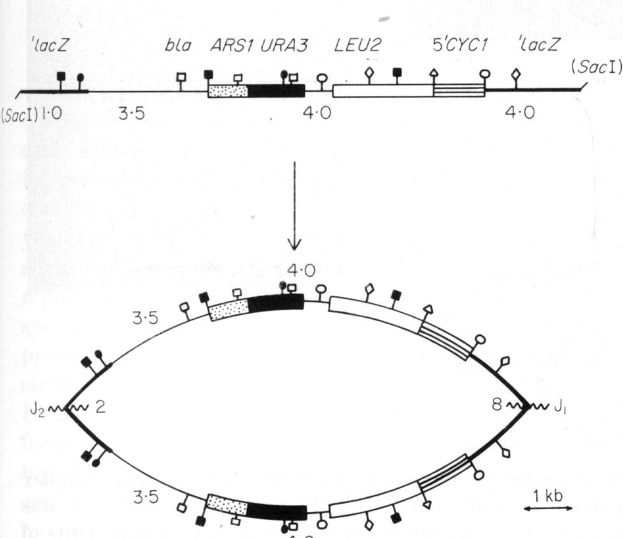
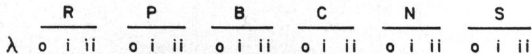


Figure 3. *Sac*I-linearized pCH308 (above the arrow) and the head-to-head (inverted) dimer product of transformation (below the arrow). *Sac*I cleavage separates the 5·0 kb *Eco*RI fragment into 1·0 kb and 4·0 kb fragments (▼, *Eco*RI site). These give rise to a 2 kb (J_2) fragment and an 8 kb (J_1) fragment upon *Eco*RI cleavage of the inverted dimer plasmid product. Wavy lines indicate the centers of apparent symmetry. Other indicated restriction sites are as follows: (♀) *Cla*I; (⇧) *Sal*I; (●) *Nco*I; (□) *Pst*I; (♀) *Bam*HI.



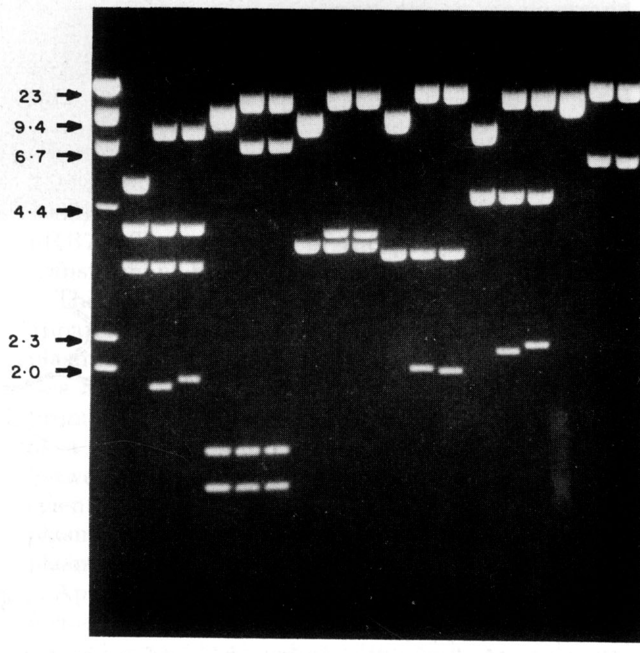


Figure 4. Restriction analysis of 2 yeast plasmids isolated in *E. coli*. DNA of plasmids I and VI (see Fig. 2), purified from *E. coli* by equilibrium banding in CsCl/ethidium bromide (as described in Materials and Methods), was digested, electrophoresed in a 1·0% agarose gel, and visualized by fluorescence after staining with ethidium bromide. A size standard of *Hin*dIII-cleaved phage λ DNA is included in the left lane, with the fragment sizes shown (in kb) on the left of the Figure. Lanes are marked as follows: 0, pCH308; i, plasmid VI; ii, plasmid I. Restriction enzyme cleavages are indicated as follows: R, *Eco*RI; C, *Cla*I; P, *Pst*I; B, *Bam*HI; N, *Nco*I; S, *Sal*I.

any one of six different restriction nucleases gave rise to all of the fragments seen with pCH308 except for the one bearing the *Sac*I site and, in addition, two fragments not present in pCH308. These new fragments were, in each case, approximately of the size expected for the junction fragments of the inverted dimer structure depicted in Figure 3. Again, apparently due to variable deletion at the end-to-end joints, the two plasmids differ slightly in the size of their comparable junction fragments.

(iii) *Electron microscopic analysis*

The putative inverted dimer plasmids isolated from *E. coli* were further examined by electron microscopy. Two of the plasmids were characterized by annealing with a derivative of pCH308 containing an insertion. Restriction nuclease *Sal*I would be expected to cleave an inverted dimer plasmid twice at symmetrical sites to yield two palindromic fragments of about 6·0 kb and 19·0 kb (Fig. 3). *Sal*I-digested inverted dimer plasmid DNA was denatured and annealed with *Sal*I-digested pCH307, a derivative of pCH308 with a 1·5 kb insert of cloned yeast *CEN5* DNA (Maine *et al.*, 1984) in the pBR322 *Bam*HI site of pCH308 (see Fig. 1). Among the hybrid structures that could be expected to form, we observed two kinds of structure containing a three-way duplex DNA joint, one of which is shown in Figure 5. This structure apparently is a heteroduplex between both strands of a *Sal*I-linearized pCH307 molecule and one strand of the 19·0 kb *Sal*I fragment of an inverted dimer plasmid. It contains in the expected positions the single strand loops due to the presence of the *CEN5* material inserted in pCH307. Furthermore, the lengths of the three duplex arms of the structure shown in the Figure would indicate that the symmetrical center of the 19·0 kb *Sal*I fragment is near the position expected for the *Sac*I site. The other structure detected that contained a three-way duplex DNA joint appeared to be a

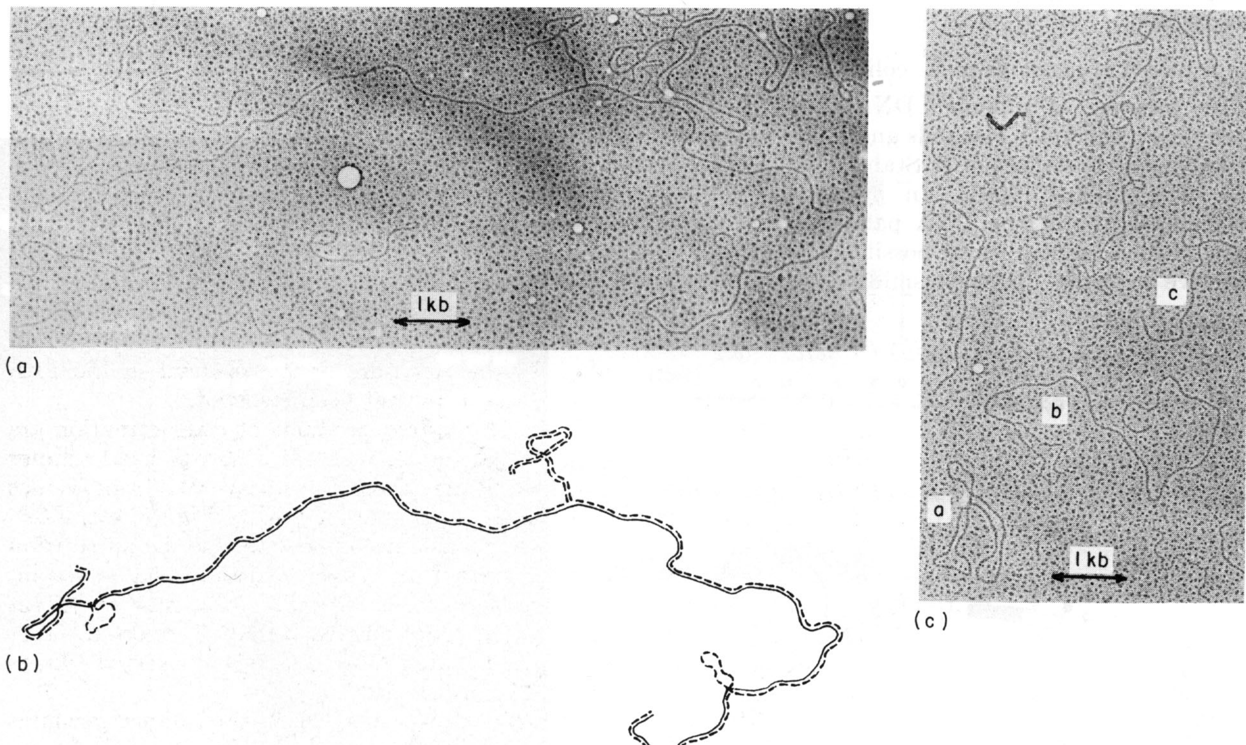


Figure 5. Electron microscopic analysis of inverted dimer plasmid products. (a) Micrograph of a heteroduplex containing a 3-way duplex DNA joint. The putative inverted dimer plasmid "IV" (Fig. 2), purified from *E. coli*, was digested with *Sal*I, which would be expected to cleave the plasmid at 2 symmetrical sites to generate 2 fragments of 6·0 kb and 19·0 kb (Fig. 3). These cleavage products were denatured and annealed with *Sal*I-cleaved pCH307, a derivative of pCH308 (Fig. 1) that contains a 1·5 kb insert of yeast *CEN5* DNA (Maine *et al.*, 1984) in its pBR322 *Bam*HI site. Electron microscopic techniques were performed as described by Davis *et al.* (1980). (b) A tracing of the heteroduplex structure shown in (a). This structure is interpreted to be a heteroduplex between both strands of a linear pCH307 molecule (broken lines) and one strand of the 19·0 kb fragment of the inverted dimer plasmid (continuous line). (c) Micrograph of stem–loop and duplex snap-back products of a partial inverted dimer plasmid. The *Sal*I cleavage products of a partial pRB73 inverted dimer plasmid (see Fig. 6 and the text) were denatured, self-annealed and spread for electron microscopic examination. The structures detected in the micrograph are identified as: a, an M13 form II molecule (size standard); b, a stem–loop molecule with an approximately 8·5 kb duplex stem and a 2·8 kb single-strand loop; c, an approximately 4·4 kb duplex DNA snap-back molecule. These structures are discussed further in the text.

heteroduplex between both strands of a *Sal*I-linearized pCH307 molecule and one strand of the 6·0 kb *Sal*I fragment of an inverted dimer plasmid.

Four of the putative inverted dimer plasmids were also characterized by denaturation and self-annealing after digestion with *Sal*I (see Fig. 3). With each plasmid DNA (plasmids I, IV, V and VI, Fig. 2), electron microscopic examination revealed two size populations of linear DNA duplexes, their average lengths approximately half of either one of the two restriction fragments produced by the cleavage. This would be expected for the snap-back structures that could form if these fragments were palindromic. Single-strand loops were not detected at the ends of any such snap-back molecules for any of the eight symmetrical junctions examined. Single-strand loops would be expected if the junctions included significant amounts (greater than several hundred base-pairs) of asymmetric sequence. Single-strand loops also could not be detected in the three-way duplex junctions of the hybrid structures formed by annealing with pCH307 (Fig. 5), as described above. These observations suggest that the end-to-end junctions are usually perfect or nearly perfect palindromes. On the other hand, the ability of these plasmids to propagate in *E. coli* suggests that the symmetrical junctions are not true palindromes. A preliminary nucleotide sequence analysis of nine of the end-to-end junctions present in these plasmid DNAs propagated in *E. coli* reveals that these junctions do not possess perfect symmetry.

(c) *Transformation with linearized plasmids bearing a 2 μm circle insert*

Plasmid pRB73, shown in Figure 1, contains a cloned segment of the yeast plasmid 2 μm circle. It also contains the cloned yeast *LEU2* gene and a fusion of the yeast *URA3* gene to *E. coli lacZ* that encodes a β-galactosidase activity expressed in yeast (Rose & Botstein, 1983*b*). The resident 2 μm segment does not contain the 2 μm plasmid replication origin but contains a site for the site-specific recombination catalyzed by the *FLP* gene product, which is encoded by the endogenous 2 μm circle plasmid (Broach & Hicks, 1980; Broach *et al.*, 1982). Plasmid pRB73 transforms yeast with a high frequency and is maintained as an autonomously replicating plasmid, presumably by incorporating a complete cellular 2 μm plasmid *via FLP*-mediated recombination (Broach & Hicks, 1980).

As in the transformation with *Sac*I-cleaved pCH308 described above, $Leu2^+$ transformants occurred frequently when pRB73 DNA, linearized by *Sac*I cleavage in the *lacZ* gene, was added to DBY1226 ($leu2^-$) spheroplasts in the presence of carrier DNA (Table 1). Nearly all of these transformants were phenotypically $lacZ^-$. Secondly, the presence during transformation of a homologous *lacZ* restriction fragment DNA which includes the *Sac*I site (see Fig. 1) stimulated the yield of $Leu2^+$ transformants (Table 1). Virtually all of these transformants were phenotypically $lacZ^+$, again as was observed in the experiment with plasmid pCH308 described above.

The $lacZ^-$ $Leu2^+$ transformants that resulted with the addition of *Sac*I-cleaved pRB73 DNA alone did not, however, contain head-to-head dimers composed of the linear pRB73 molecules. Gel-transfer hybridization analysis of DNA from 14 of these transformants, and restriction and electron microscopic analyses of plasmids recovered in *E. coli*, indicate that the predominant plasmid product has the more complex structure depicted in Figure 6 (structure V). This structure, observed in 13 of the 14 transformants, is a partial dimer of the linearized plasmid molecule. It contains a single symmetrical junction about which sequence symmetry extends 12·9 kb to include two 599 base-pair inverted repeat (IR) sequences of 2 μm circle. Between these IR sequences is 2·8 kb of unique sequence. This is demonstrated by the formation of stem–loop molecules upon denaturing and self-annealing the *Sal*I cleavage products of purified plasmid DNA (Fig. 5). The two predominant structures detected by electron microscopy are duplex molecules of about half the length of the 8·8 kb *Sal*I fragment, which putatively contains the symmetrical junction, and stem–loop molecules with an 8·5 kb duplex stem and a single-strand loop of approximately 2·8 kb. These observations and restriction analysis of these plasmids indicate that the 2·8 kb unique sequence is the large (2774 base-pairs) unique region of the 2 μm circle plasmid (Hartley & Donelson, 1980) which contains the 2 μm circle replication origin (Broach & Hicks, 1980), a DNA segment that is not present in pRB73. The structure of the plasmid in the 14th transformant has not been resolved.

These 13 plasmid products of transformation are apparently derivatives of head-to-head dimer plasmids formed from the linear pRB73 molecules (see Fig. 6). As is described in the Figure, two *FLP*-promoted recombination events, one the integration of a cellular 2 μm circle plasmid, and a second between two *FLP* recombination sites in direct orientation, could resolve a pRB73 inverted dimer plasmid, yielding the "partial" inverted dimer plasmids that are detected.

Apparent derivatives of inverted dimer plasmids formed *via FLP*-promoted recombination have been observed in transformation with other plasmid DNAs and with pRB73 cleaved at sites other than the *Sac*I site in the *lacZ* gene. These were observed with *Bam*HI-digested pRB30 (Falco *et al.*, 1982), and with *Bam*HI-digested plasmid CV6 (Broach & Hicks, 1980), cleavages which create plasmid ends in the pBR322 sequence. When pRB73 (Fig. 1) was digested with either *Bam*HI, or both *Bam*HI and *Sac*I, the $Leu2^+$ transformants analyzed were also found to contain the expected *FLP*-promoted derivatives of an inverted dimer plasmid. These repair events involve linear molecules with a *URA3* and a *lacZ* end. A similar result was obtained with

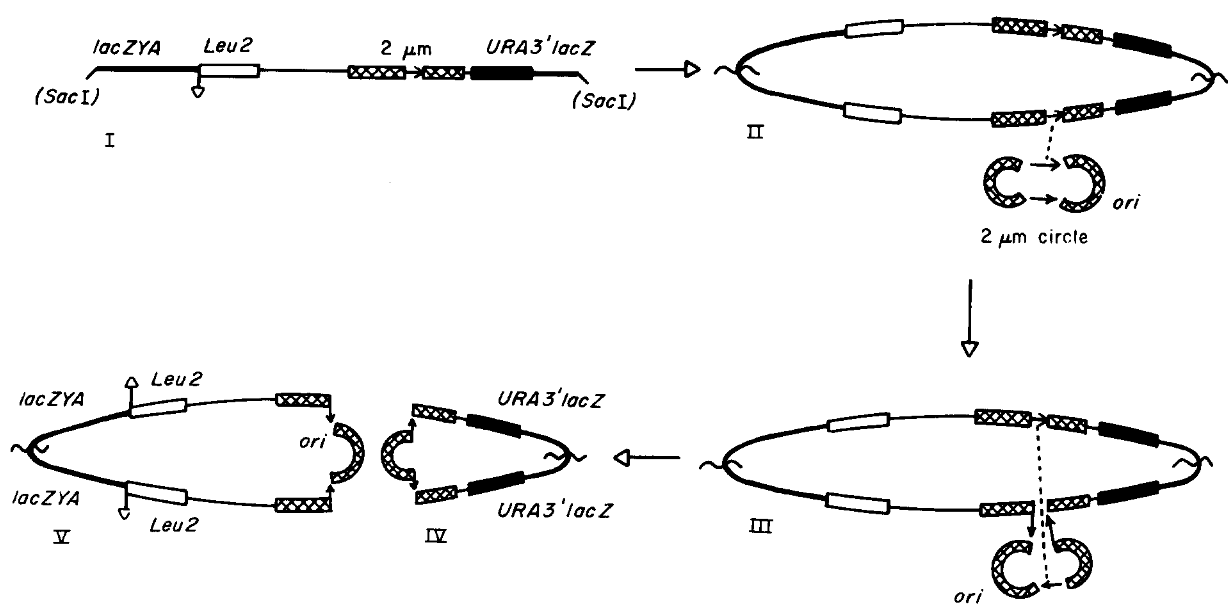


Figure 6. The plasmid product of transformation with *Sac*I-cleaved pRB73 and a possible mechanism for its formation. Structure II, the head-to-head dimer plasmid that could be formed from the pRB73 linear molecules (I), contains two sites for *FLP*-mediated site-specific recombination (arrows indicate the positions of the 2 μm plasmid 599 base-pair inverted repeat (IR) sequences, which contain the site of *FLP*-mediated recombination; Broach *et al.*, 1982). The inverted dimer plasmid would thus be expected to be a substrate for the integration of a cellular 2 μm circle plasmid, as shown in the Figure (broken lines indicate sites involved in a *FLP* recombination event). The proficiency of this recombination system makes this event likely, but it also appears to be essential for replication of a plasmid bearing the 2 μm plasmid segment present in pRB73, as discussed in the text. The product formed by this integration event (structure III) would be expected to be unstable because it contains 2 *FLP* recombination sites in direct orientation. An exchange between these sites would resolve the molecule into the 2 products labeled IV and V. Product V, which contains 2 *LEU2* genes and the 2 μm replication origin (*ori*), is the plasmid detected among 13 of the 14 transformants investigated and is described further in the text as a "partial" inverted dimer plasmid. Product IV, which has not been observed, would not be maintained by the selection. Its loss is therefore not surprising. An open arrow (⇧) indicates the position of *Sal*I restriction sites.

pRB73 DNA cleaved with *Bcl*I, which generates plasmid DNA ends in the *lacZ* and *lacA* genes.

(d) *Dependence of inverted dimer formation on the presence of sonicated non-homologous carrier DNA during transformation*

In the transformation experiments described above, yeast spheroplasts were exposed to linearized plasmid DNA along with sonicated chicken erythrocyte (carrier) DNA (as described in Materials and Methods). When this carrier DNA was not included during transformation, the yield of transformants with closed circular or *Sac*I-linearized plasmid DNA (pCH308, Fig. 1) was reduced as much as 100-fold; the extent of this reduction lessened at higher plasmid DNA concentrations. When tested on X-gal indicator plates, 20 to 50% of the $Leu2^+$ transformants obtained with linearized plasmid DNA were found to be $lacZ^+$, depending upon the DNA concentration during the transformation. All of 24 of the $lacZ^-$ $Leu2^+$ transformants examined by gel-transfer hybridization analysis were found to harbor monomer plasmids with a deletion encompassing the *Sac*I site in *lacZ*. Inverted dimer plasmids were not detected. This outcome of transformation with a linearized plasmid appears similar to that reported by Orr-Weaver & Szostak (1983) and Suzuki *et al.* (1983).

There appear to be two separable activities of carrier DNA in transformation. With the presence of phage φX174 RFI DNA as carrier, the yield of transformants with *Sac*I-linearized pCH308 is modestly increased over the yield obtained in the absence of carrier DNA. Yet, as in the absence of carrier DNA, the transformants are found to harbor either parental plasmids or plasmids with a deletion encompassing the *Sac*I site. When the φX174 RFI carrier DNA has been linearized by *Pst*I cleavage and then sonicated, the yield of transformants is more substantially increased than with intact φX174 RFI DNA. Examination of these transformants by gel-transfer hybridization reveals that the formation of inverted dimer plasmids can account for most of the increased yield. Phage λ DNA cleaved with *Rsa*I (which generates 114 fragments) can also promote the formation of inverted dimer plasmids. These observations suggest that free DNA ends may be active in promoting the formation of inverted dimers.

4. Discussion

In the experiments reported here, the predominant plasmid product of transformation

with a plasmid DNA, cleaved to generate free ends lacking homology with the yeast genome, was a head-to-head (inverted) dimer of the linearized plasmid molecule. In the experiment with linearized plasmid pCH308 described in Table 1, inverted dimer plasmids were detected in 82% of the transformants. In the experiment with linearized plasmid pRB73, 93% of the transformants contained "partial" inverted dimer plasmids, apparent derivatives of inverted dimer plasmids formed *via FLP* site-specific recombination (Fig. 6). The presence of sonicated carrier DNA during transformation appears to account for the difference between this novel outcome of transformation and the previously reported formation of monomer products. We find that in the absence of carrier DNA, transformants occur at a much reduced frequency and contain monomer products; plasmids indistinguishable from the parent plasmid or plasmids with deletion at the site of the cleavage. The influence of carrier DNA on the outcome of transformation is discussed further below.

A repair mechanism in which the molecular ends of a linearized plasmid are randomly joined could be expected to give rise to monomer plasmids and, perhaps, to multimers consisting of linears joined in the head-to-head and head-to-tail orientations. In the formation of multimers, head-to-head joints could be expected to form as often as head-to-tail joints. The monomer product of re-circularization (Orr-Weaver & Szostak, 1983), and plasmids formed by the joining of multiple restriction fragments (Suzuki *et al.*, 1983) could be accounted for as products of a mechanism in which plasmid ends join randomly. The predominance of inverted dimer plasmid formation when carrier DNA is present might reflect the preferred joining of particular ends or the relatively poor viability of the products of head-to-tail joinings. The latter explanation, though it cannot be excluded, seems unlikely.

A preference for the joining of particular ends (head-to-head as opposed to head-to-tail) could be a property of the specific DNA sequences at these plasmid's *lacZ* ends. Such a preference would have to be extreme in order to account for the predominance of inverted dimer formation in transformation at sub-saturating concentrations of linearized plasmid DNA, where monomeric re-circularization should be favored. Furthermore, the formation of inverted dimer plasmids is not a special property of linear molecules bearing *Sac*I-generated *lacZ* ends. Transformation with linearized plasmids bearing *Bcl*I-generated ends in the *lacZ* and *lacA* genes, or with ends within the pBR322 or *URA3* gene sequence, results in an outcome similar to that with *Sac*I-linearized pRB73, a predominance of "partial" inverted dimer plasmids among the transformants. The observed variable deletion of material in the symmetrical junctions also suggests that this outcome of transformation is not a characteristic of special sequences at a plasmid's molecular ends. If the junctions that we detect were the original products of the joining event, joining would have occurred at any of the numerous sites near the end originally introduced by enzme cleavage.

An alternative explanation for the predominant recovery of inverted dimer plasmids is that yeast possesses a DNA double strand break-processing mechanism whose product is a symmetrical joint. Mechanisms of DNA double strand break repair that result in a symmetrical junction at the original site of a free DNA end have been described. Yeast respiration-deficient (rho^-) mutants, readily induced with mutagens that result in mitochondrial DNA double strand breaks, are often found to contain head-to-head duplications of mitochondrial DNA (Locker *et al.*, 1974; Sor & Fukuhara, 1983). In maize, a mechanism for the processing of chromosome breaks involves the apparent fusion of the daughters of a broken chromosome at their free DNA ends (McClintock, 1939). Genetic experiments may have revealed such a process in yeast (Haber *et al.*, 1984). The formation of head-to-head λdv plasmids, while not known to involve a double strand break, may be an example of a prokaryotic mechanism of forming symmetrical joints (Berg, 1974; Chow *et al.*, 1974).

The mechanism of inverted dimer plasmid formation might involve the fusion of two linear plasmid molecules or the replication of a single linear molecule. For example, joining of the 5′ strand to the 3′ strand at each free end of a linear duplex molecule would result in a hairpin molecule that, upon replication, could yield an inverted dimer plasmid. Alternatively, two input linear duplexes, or the daughter replicons of a single linear molecule, might pair by homology, thus bringing into proximity the molecular ends that are fused. Preliminary experiments indicate that inverted dimer formation occurs *via* a bimolecular mechanism (our unpublished results; Kunes *et al.*, 1984).

This putative DNA repair mechanism is detected during transformation only when sonicated carrier DNA is present. The reason for this is presently not clear. Whereas closed circular molecules (phage ϕX174 RFI DNA) do not promote the formation of inverted dimers, small DNA fragments (phage λ DNA cleaved with *Rsa*I) do. This observation would suggest that a high concentration of free DNA ends, which apparently is not achieved by the presence of the linearized plasmid DNA alone, is the requirement for inverted dimer formation satisfied by the presence of sonicated carrier DNA.

Carrier DNA is apparently not incorporated into the symmetrical junctions. A preliminary DNA sequence analysis of nine end-to-end plasmid junctions revealed that they are composed solely of DNA sequence present in the parent plasmid (pCH308). This does not rule out the possibility of a role of carrier DNA as a transient component of the junction. Some other potential explanations for the role of carrier DNA are that free DNA ends act as a cofactor in the junction-forming mechanism, or that

a high concentration of free DNA ends masks the activity of a cellular nuclease that destroys an intermediate in the pathway of symmetrical junction formation. Perhaps a modulator of a cellular response to DNA damage is bound by free DNA ends, signaling for the expression of an enzyme that catalyzes symmetrical junction formation, or activating this enzyme directly.

When *lacZ* restriction fragment DNA is included along with plasmid DNA cleaved by *Sac*I in its resident *lacZ* gene, the result of transformation in the presence of sonicated carrier DNA is the formation of plasmids with the parental plasmid structure. Inverted dimer plasmids are not detected. This outcome is apparently due to homologous recombination, as is shown by the high frequency of marker rescue when the linearized plasmid and homologous fragment are genetically marked. Furthermore, at sub-saturating concentrations of the linearized plasmid DNA, there is a marked increase in the frequency of transformants when the *lacZ* fragment is present. This suggests that the recombinational repair is highly efficient, which is consistent with the high efficiency of chromosomal double strand break repair in yeast (Resnick, 1976) and the high frequency of plasmid, chromosome recombination observed with a plasmid cleaved in a region of chromosomal homology (Orr-Weaver *et al.*, 1981; Orr-Weaver & Szostak, 1983).

If an independent population of products had been created by homologous recombination, then 10 to 50% of the transformants, depending on the particular concentration of linearized plasmid DNA during the transformation, could be expected to harbor an inverted dimer plasmid. However, transformants harboring these plasmids are not detected. This would suggest that the presence of the *lacZ* fragment DNA suppresses the formation of inverted dimers. An explanation of this phenomenon might be that inverted dimer formation is both relatively inefficient and, as stated, apparently bimolecular. As is depicted in Figure 7, efficient homologous pairing with an intact restriction fragment substrate could remove either of the two linear plasmid substrates necessary for inverted dimer formation, and yield a monomer circle by recombinational repair. This apparent relationship between homologous recombination and a novel DNA transaction that yields rearrangement could be important, as the latter outcome in the processing of genomic double strand breaks could often lead to loss of gene function and lethality.

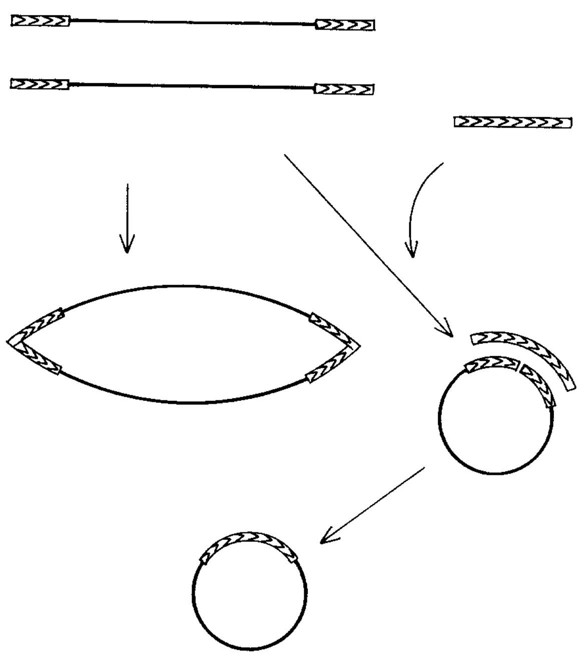


Figure 7. Formation of inverted dimer plasmids and recombinant plasmids. Two linear plasmid molecules may fuse to form an inverted dimer plasmid. However, efficient homologous pairing with an intact restriction fragment substrate (open box with arrow heads) removes either linear plasmid molecule as a substrate for inverted dimer formation, and yields a monomer circle by recombinational repair.

We thank Micheal Lichten, Mark Rose, Karen Overbye and Connie Holm for their advice and ideas and for providing plasmid DNA.

References

Bach, M. L., Lacroute, F. & Botstein, D. (1979). *Proc. Nat. Acad. Sci., U.S.A.* **76**, 386–390.

Beggs, J. D. (1978). *Nature (London)*, **275**, 104–109.

Berg, D. (1974). *J. Mol. Biol.* **86**, 59–68.

Bolivar, F., Rodriguez, R. L., Green, P. J., Betlach, M. C., Heyneker, H. L., Boyer, H. W., Crosa, J. H. & Falkow, S. (1977). *Gene*, **2**, 95–113.

Boyer, H. W. & Roulland-Dussoix, D. (1969). *J. Mol. Biol.* **41**, 459–472.

Broach, J. R. & Hicks, J. B. (1980). *Cell*, **21**, 501–508.

Broach, J. R., Guarascio, V. R. & Jayaram, M. (1982). *Cell*, **29**, 227–234.

Chow, L., Davidson, N. & Berg, D. (1974). *J. Mol. Biol.* **86**, 69–89.

Clewell, D. B. & Helinski, D. R. (1969). *Proc. Nat. Acad. Sci., U.S.A.* **62**, 1159–1166.

Collins, J. (1981). *Cold Spring Harbor Symp. Quant. Biol.* **45**, 409–416.

Davis, R. W., Botstein, D. & Roth, J. R. (1980). *Advanced Bacterial Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor.

Falco, S. C., Li, Y., Broach, J. R. & Botstein, D. (1982). *Cell*, **29**, 573–584.

Gellert, M., Mizuuchi, K., O'Dea, M. H., Ohmori, H. & Tomizawa, J. (1979). *Cold Spring Harbor Symp. Quant. Biol.* **43**, 35–40.

Guarente, L. & Ptashne, M. (1981). *Proc. Nat. Acad. Sci., U.S.A.* **78**, 2199–2203.

Haber, J. E., Thorburn, P. C. & Rogers, D. (1984). *Genetics*, **106**, 185–205.

Hartley, J. L. & Donelson, J. E. (1980). *Nature (London)*, **286**, 860–864.

Hicks, J. B., Hinnen, A. & Fink, G. R. (1979). *Cold Spring Harbor Symp. Quant. Biol.* **43**, 1305–1313.

Hinnen, A., Hicks, J. B. & Fink, G. R. (1978). *Proc. Nat. Acad. Sci., U.S.A.* **75**, 1929–1933.

Kunes, S., Botstein, D. & Fox, M. S. (1984). *Cold Spring Harbor Symp. Quant. Biol.* **49**, 617–628.

Leach, D. R. F. & Stahl, F. W. (1983). *Nature (London)*, **305**, 448–451.

Locker, J., Rabinowitz, M. & Getz, G. S. (1974). *Proc. Nat. Acad. Sci., U.S.A.* **71**, 1366–1370.
Maine, G. T., Surosky, R. T. & Tye, B. K. (1984). *Mol. Cell. Biol.* **4**, 86–91.
McClintock, B. (1939). *Proc. Nat. Acad. Sci., U.S.A.* **25**, 405–416.
Miller, J. H. (1972). *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor.
Orr-Weaver, T. L. & Szostak, J. W. (1983). *Proc. Nat. Acad. Sci., U.S.A.* **80**, 4417–4421.
Orr-Weaver, T. L., Szostak, J. W. & Rothstein, R. J. (1981). *Proc. Nat. Acad. Sci., U.S.A.* **78**, 6354–6358.
Page, D. C. & de la Chapelle, A. (1984). *Amer. J. Hum. Genet.* **36**, 565–575.
Radloff, R., Bauer, W. & Vinograd, J. (1967). *Proc. Nat. Acad. Sci., U.S.A.* **57**, 1514–1521.
Rambach, A. & Hogness, D. S. (1977). *Proc. Nat. Acad. Sci., U.S.A.* **74**, 5041–5045.
Ratzkin, B. & Carbon, J. (1977). *Proc. Nat. Acad. Sci., U.S.A.* **74**, 487–491.
Resnick, M. A. (1976). *J. Theoret. Biol.* **59**, 97–106.
Resnick, M. A. & Martin, P. (1976). *Mol. Gen. Genet.* **143**, 119–129.
Rose, M. & Botstein, D. (1983*a*). *Methods Enzymol.* **101**, 167–180.
Rose, M. & Botstein, D. (1983*b*). *J. Mol. Biol.* **170**, 883–904.
Rose, M., Casadaban, M. J. & Botstein, D. (1981). *Proc. Nat. Acad. Sci., U.S.A.* **78**, 2460–2464.
Sherman, F., Fink, G. R. & Lawrence, C. W. (1979). *Methods in Yeast Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor.
Southern, E. M. (1975). *J. Mol. Biol.* **98**, 503–517.
Sor, F. & Fukuhara, H. (1983). *Cell*, **32**, 391–396.
Struhl, K., Stinchcomb, D. T., Scherer, S. & Davis, R. W. (1979). *Proc. Nat. Acad. Sci., U.S.A.* **76**, 1035–1039.
Suzuki, K., Imai, Y., Yamashita, I. & Fukui, S. (1983). *J. Bacteriol.* **155**, 747–754.

Edited by I. Herskowitz