Transformation of Yeast with Linearized Plasmid DNA
Formation of Inverted Dimers and Recombinant Plasmid Products

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The molecular products 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 & Steitz, 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 & Steitz, 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

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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 (Broyer & Roulland-Dussoux, 1969) was used in the construction and preparation of plasmids pSK117, pSK120 and pSK127. Strain HB101 was also the recipient of plasmid recovered from yeast transformation. Yeast strain DBY1286 (MATa leu2-3 leu2-112 trp1-1 ura3-1) 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 (M.I.T.).

(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 (1985a).

(c) DNA preparation and cleavage

Plasmid DNA was isolated from E. coli by a modification (Rambach & Hoggness, 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). Supercopied plasmid DNA was stored in TE buffer (10 mM Tris, pH 8.0, 1 mM NaCl, EDTA) 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 10⁶ cells grown in SD liquid medium (with selection when appropriate).

Chicken erythrocyte DNA was obtained from Caltech-Behring (La Jolla, CA). After dissolution in TE buffer, it was sonicated to an average molecular weight of 5 x 10⁷, 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 x 10⁸. DNA of RHI 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

STAT buffer (1 M sorbitol, 10 mM-Tris, pH 7.5, 10 mM CaCl₂) was substituted for 1 M sorbitol in the third wash after treatment with glycerol (DuPont Pharmaceuticals, Wilmington, DE). The final suspension contained spheroplasts at a concentration of about 10⁶/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 presence 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 putative 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 (Guarante & Ptashne, 1981) that encodes an β-galactosidase activity expressed in yeast. The plasmid is cleaved once by restriction nuclease SacI 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 SacI-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 SacI site, and hence might serve as a homologous substrate for the repair of the SacI-cleaved plasmid, was inserted into the vector pBR322 (Bolivar et al., 1977). This fragment (Fig. 1) extends 1.9 kb and 1.1 kb to the BamHI and EcoRI sites, respectively, on either side of the SacI site.

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

Though SacI-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 into non-homologous joining events described by Orr-Weaver & Szostak (1983) and Suzuki et al. (1983).

Abbreviations used: kb, 10³ bases or base-pairs; IR, inverted repeat(s).

Their observation often rests on circular non-replicating DNA, though pBR322 is linear. Hence, its lacZ coding sequence is the linearized transformant (see below for expected I. transformation of the sequence.)

(ii) Transformant formation in the presence of a homologous DNA fragment

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A plasmid bearing the closed yeast LEU2 and URA3 genes and capable of autonomous replication in yeast is shown in Figure 1. This plasmid, pCH208, contains a fusion of the Saccharomyces CUC1 gene to the E. coli lacZ gene (Garner et al., 1981) that encodes a β-galactosidase activity expressed in yeast. The plasmid is cleaved once by restriction nuclease Sst1 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 Sac1-cleaved pCH208 would not be expected to be appropriate substrates for homologous recombination with sequences present in the yeast genome. A restriction fragment of pCH208 that includes the Sac1 site, and hence might serve as a homologous substrate for the repair of the Sac1-cleaved plasmid, was subcloned into the vector pBR322 (Bolivar et al., 1977). This fragment (Fig. 1) extends 1.9 kb and 1.4 kb to the BamHI and EcoRI sites, respectively, on either side of the Sac1 site.

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

Though Sac1-cleaved pCH208 would not be expected to be repaired by homologous recombination with a yeast chromosomal locus, transforms could be expected with this DNA since the linear molecules might circulate via the non-homologous joining events described by Weaver & Szostak (1983) and Suzuki et al. (1983). Their observations suggest that these events would often restore the Sac1 site and the lacZ coding sequence. Such transforms should express β-galactosidase, which is easily assayed on minimal medium containing the chromogenic substrate X-gal (Garner & Plaschke, 1981; Ros et al., 1981). As shown in Table 1, Lac+ transforms do result from exposure of the leu2 strain yeast, DBY1220, to sonicated carrier DNA and various amounts of Sac1-cleaved pCH208 DNA. These transforms occur at a frequency that is from 2% to 5% that observed with an equivalent amount of closed circular pBR328 DNA. In contrast to the transforms obtained with the closed circular DNA, most of those obtained with the linearized plasmid DNA are prototrophically lacZ- (Table 1).

Hence, few of these transforms have restored the lacZ coding sequence which had been cleaved by Sac1. 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 transforms is explored below.

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

The outcome is significantly different when the BamHI, EcoRI restriction fragment of lacZ is
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

<table>
<thead>
<tr>
<th>Plasmid DNA added (µg)</th>
<th>pCH308</th>
<th>pBR322</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>250</td>
</tr>
<tr>
<td><em>lacz</em></td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td><em>lacZ</em></td>
<td>0.02</td>
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<tr>
<td><em>lacZ</em></td>
<td>0.02</td>
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<tr>
<td><em>lacZ</em></td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Transformation of yeast strain DBY1226 (wild-type lacZ) was carried out in the presence of sonicated chicken rRNA (carrier) DNA as described in Materials and Methods. The frequencies of *lacZ* transformants shown in the Table are normalized to 1000 (closed circle pCH308 at 3000 µg or closed circular pBR322 at 3000 µg), and are about 10^(-7) the actual yield. In these cases in which the unselected *lacZ* fragment was provided, about 2 µg of the appropriate pBR322 vector (see Fig. 1), cleaved to liberate the fragment from the vector sequence (BamHI, EcoRI-digested pBR322 for pCH308 and HindIII, EcoRI-digested pBR322 for pBR322) were added along with the SacI-linearized plasmid and carrier DNA. Transformsants were tested for their *lacZ* phenotype on yeast minimal medium containing the indicator X-Gal in the absence of inositol. Plates that remained white after several days at 30°C were scored as *lacZ*; *lacZ* transformants gave rise to a dark blue patch. Transformsants with intermediate phenotypes were rare (less than 1%).

Table 2  
Marker rescue associated with the *lacZ* fragment-deleted linearized plasmid

<table>
<thead>
<tr>
<th></th>
<th><em>lacZ</em></th>
<th><em>lacZ</em></th>
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<tbody>
<tr>
<td></td>
<td>4200</td>
<td>0/12</td>
</tr>
<tr>
<td><em>lacZ</em></td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td><em>lacZ</em></td>
<td>1700</td>
<td>5/13</td>
</tr>
</tbody>
</table>

Transformation of strain DBY1226 in the presence of wild-type described in Materials and Methods. Approximately 100 µg of plasmid pBR322 (Fig. 1) described in the text, were added to sonicated chicken rRNA or in the closed circular (co) form. When provided, appropriate pBR322 DNA, cleaved with BamHI and EcoRI, or with EcoRI-hydrolyzed fragment insert (see Fig. 1), were added as a mixture with the pBR322 (wild-type) fragment subcloned in pK117 (denoted as a shorter one even than the pBR322 fragment subcloned in pK120). Transformation with *lacZ* transformants and the fraction of trans- 

substrates. We utilized a mutant derivative of the *URA3*/*lacZ* fusion plasmid, pBR73 (Rose & Botstein, 1976), shown in Figure 1. The mutant plasmid, pK117, 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 *SacI* linker at the site of the *URA3* gene fusion with *lacZ*. The wild-type start codon is included in the HindIII-EcoRI fragment of pBR73 subcloned in pK120 (see Fig. 1), but is not included in the 272 base pair shorter HindIII-EcoRI fragment subcloned in pSK117. Both subcloned fragments include the *SacI* site in the plasmid *lacZ* sequence.

Transformation of the *lacZ* strain DBY1226 was carried out with the SacI-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 *lacZ* 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

The *lacZ* fragment

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<tr>
<td></td>
<td>4200</td>
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</tr>
<tr>
<td><em>lacZ</em></td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td><em>lacZ</em></td>
<td>1700</td>
<td>5/13</td>
</tr>
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<table>
<thead>
<tr>
<th>Plasmid (2A added pg)</th>
<th>pBR322</th>
<th>pBR321</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>250</td>
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</tr>
<tr>
<td>500</td>
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<td>2500</td>
<td>2500</td>
</tr>
<tr>
<td>5000</td>
<td>5000</td>
<td>5000</td>
</tr>
</tbody>
</table>

Table 2
Formation of Inserted Dimers in Yeast

<table>
<thead>
<tr>
<th>Insertion</th>
<th>Lacs +</th>
<th>LacZ +</th>
<th>LacZ +/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4200</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>1 lacZ</td>
<td>200</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>1 lacZ + 1 insert</td>
<td>2000</td>
<td>0/145</td>
<td></td>
</tr>
<tr>
<td>1 lacZ + 1 insert + 1 lacZ</td>
<td>1700</td>
<td>7/10</td>
<td></td>
</tr>
</tbody>
</table>

Transformation of strain DBY1298 in the presence of nitroreductase DNA was carried out as described in Materials and Methods. Approximately 100 μg of plasmid pK0111, a mutant derivative of pBR373 (Fig 5) described in the text, was added to appropriate bacterial culture with SceI and in the closed circular (rc) form. When present, approximately 5 μg of either pH115 DNA or pK120 DNA, cleaved with SacI and EcoRI, or with HindIII and EcoRI, respectively, to liberate the fragment insert (see Fig 1) was added as a mixture with the linearized mutant plasmid. The pBR373 (wild-type) fragment subcloned in plasmid pK117 (inserted in the Table as 2 lacZ + lacZ) was sequenced by the method of Sanger and Smith (1977). Each of these was found to contain an apparent plasmid derivative of pBR380 with a deletion encompassing the SacI site. These deletions presumably account for their lacZ phenotype.

The lacZ fragment with SacI-cleaved pCH308. The lacZ phenotype of the LacZ + transforms was then tested on X-gal indicator plates. Those obtained with SacI-linearized pK0111 alone were all white (lacZ−). Most of those obtained with the combination of linearized pK0111 and SacIHI, EcoRI-cleaved pK117 (the shorter fragment) showed the original pK0111 lacZ mutant phenotype (pale blue), consistent with their having undergone homologous fragment-dependent repair. Approximately 55% of these LacZ + transforms obtained with pK0111 in combination with HindIII, EcoRI-cleaved pK120 (the longer fragment) were fully lacZ + (dark blue), while most of the remainder showed the pale blue pK0111 phenotype. These lacZ + plasmids have apparently inherited the restriction fragment marker. If the digested pK120 DNA was provided along with pK0111 in closed circular form, only 1% of the LacZ + transforms 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 + LacZ + transforms, the predominant class obtained with SacI-cleaved pCH308
DNA in the absence of the homologous lacZ fragment, were examined for instability of the
LeuZ2 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 LeuZ2 phenotype. This instability is characteristic of a plasmid that, like
pCH308, is rendered autonomous by a resident yeast ARN1 segment (Struhl et al., 1979).

The leuZ-LeuZ 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 EcoRI-digested yeast DNA from 24 leuZ-LeuZ transformants
with a probe containing pBR322, lacZ and URA1 sequences (see Fig. 2) gave the following result. One
transformant had no detectable plasmid sequence. Two transformants lacked the SacI site-containing
5-0 kb EcoRI fragment of pCH308, had the other two pCH308 EcoRI fragments (3-5 kb and 4-6 kb)
and bore a restriction fragment not present in pCH308 that was somewhat smaller than the EcoRI
fragment containing the SacI site. These two transformants probably contain pCH308
derivatives with a deletion encompassing the SacI site, a kind of repair product that has been reported
(Suzuki et al., 1985). For the remaining 21 transformants, the SacI site-containing EcoRI
fragment was absent, the two other EcoRI fragments of pCH308 were present, and two new
restriction fragments of approximately 2 kb and 8 kb were detected. A structure that can account
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,
head-to-head (inverted) dimer of the SacI-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 J1 and J2 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 SacI-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 J2 of plasmid
HI in Fig. 2). We suppose that material at the symmetrical joint is to a variable extent deleted. The absence of a SacI 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 φ1 phage inviable
in an E. coli host (Leach & Stahl, 1983; Gellert et al., 1979; Collins, 1981). In yeast, however, a
plasmid containing a true DNA palindrome is viable (unpublished result). It is possible then that the
putative inverted dimer plasmids detected in the

Figure 2. Gel-transfer hybridization of EcoRI-cleaved DNA from yeast and E. coli transformants. EcoRI-cleaved
DNA of yeast strain DBY1290, pCH308 (R, purified from E. coli strain HB101, K, additional purification by binding
in a CsCl/Ethidium bromide gradient; S, DNA from transformed DBY1290, 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, URA1 and lacZ sequences detects the 14 kb URA1
fragment of yeast (lanes marked S), and the EcoRI fragments of pCH308. Plasmids I through VI are isolated from
transformants obtained with SacI-linearized pCH308 DNA, as described in the text. J1 and J2 denote the 2 new
fragments (absent in pCH308) that appear to be the junction-containing fragments of an inverted dimer (see Fig. 3).
Plasmid HI appears to be present in yeast at a low copy number, and hence the plasmid bands are faint at the film
exposure time shown.

Figure 3. SacI-linearized pCH308 (above the arrow) and the head-to-head (inverted) dimer product of transformation (below the arrow). SacI cleavage separates the 5-0 kb EcoRI fragment into 4-6 kb and 3-5 kb fragments (EcoRI site). These give rise to a 2 kb (J2 fragment) and an 8 kb (J1 fragment) upon EcoRI cleavage of the inverted dimer plasmid product. Wavy
lines indicate the centers of apparent symmetry. Other indicated restriction sites are as follows: (T) ClaI; (E) SaII; (N)
NcoI; (P) PstI; (F) BamHI.

Figure 4. Gel-isolated in E. coli, plasmid pCH308 purified from ethidium bromide-treated agarose gel, and
with ethidium bromide-digested plasmid fragments of pCH308. Lanes are marked as follows: I, pCH308; II, pCH308
NcoI; III, pCH308 EcoRI; IV, pCH308 BamHI.
(i) Gel-transfer hybridization

Gel-transfer hybridization of EcoRI-digested yeast DNA from 24 lacZ-Leu2α 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 SacI site-containing 5-kb EcoRI fragment of pCH308, had the other two pCH308 EcoRI fragments (3.5 kb and 4.6 kb) and bore a restriction fragment not present in pCH308 that was somewhat smaller than the EcoRI fragment containing the SacI site. These two transformants probably contain pCH308 derivatives with a deletion encompassing the SacI site, a kind of repair product that has been reported (Struski et al., 1983). For the remaining 21 transformants, the SacI site-containing EcoRI fragment was absent, the two other EcoRI fragments of pCH308 were present, and two new restriction fragments of approximately 2 kb and 8 kb were detected. A structure that can account 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 SacI 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 J1 and J2 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 SacI-generated molecular ends. Most of the junction fragments are 90 to 300 base-pairs smaller, though, occasionally a junction fragment is missing more than 1000 base-pairs (see, for example, fragment J2 of plasmid III in Fig. 2). We suppose that material at the symmetrical joint is to a variable extent deleted. The absence of a SacI 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 palindromic sequence has been shown to render plasmids and 3 phage inviable in E. coli host (Leach & Stahl, 1983; Gelbert et al., 1979; Collins, 1981). In yeast, however, a plasmid bearing a true DNA palindromic 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 (Baggs, 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 EcoRI 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

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Figure 3. SacI-linearized pCH308 (above the arrow) and the head-to-head (inverted) dimer product of transformation (below the arrow). SacI cleavage separates the 5-kb EcoRI fragment into 1.4-kb and 4.6-kb fragments. The EcoRI site, 4.4-kb fragment. These give rise to a 2-kb (J1) fragment and an 8-kb (J2) fragment upon EcoRI cleavage of the inverted dimer plasmid product. Wavy lines indicate the centers of apparent symmetry. Other indicated restriction sites are as follows: (a) CalII, (b) SallI, (c) XhoI, (d) PstI, (e) BamHI.

Figure 4. Restriction analysis of 2 yeast plasmids isolated in E. coli. DNA of plasmid 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 1.4% agarose gel, and visualized by fluorescence after staining with ethidium bromide. A site standard of HindIII-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: EcoRI, G: CalII, P: PstI, B: BamHI, N: NdeI, S: SallI.
any one of six different restriction nuclease gav
rise to all of the fragments seen with pCH308 except for the one bearing the SacI site and, in addition, two fragments not present in pCIH308. 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 pCIH308 containing an insertion. Restriction nuclease SacI would be expected to cleave an inverted dimer plasmid twice at symmetrical sites to yield two palindromic fragments of about 60 kbp and 190 kbp (Fig. 3). SacI-digested inverted dimer plasmid DNA was denatured and annealed with SacI-digested pCIH307, a derivative of pCIH308 with a 1-kbp insert of cloned yeast CEN5 DNA (Maine et al., 1984) in its pBR322 BamHI site. The hybrid structures could be expected to form 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 SacI-linearized pCIH307 molecule and one strand of the 190-kbp SacI 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 pCIH307. Furthermore, the lengths of the three duplex arms of the structure shown in the Figure would indicate that the symmetrical center of the 190-kbp SacI fragment is near the position expected for the SacI site. The other structure detected that contained a three-way duplex DNA joint appeared to be a heteroduplex between both strands of a SacI-linearized pCIH307 molecule and one strand of the 60-kbp SacI fragment of an inverted dimer plasmid.

Four of the putative inverted dimer plasmids were also characterized by denaturation and self-annealing after digestion with SacI (see Fig. 3). With each plasmid DNA (plasmids I, IV, V and VI, Fig. 2), electron microscopic examination revealed two site 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 could not be detected in the three-way duplex junctions of the hybrid structures formed by annealing with pCIH307 (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 pBR73, shown in Figure 1, contains a cloned segment of the yeast plasmid 2 μm circle. It also contains the cloned yeast LEB7 gene and a fusion of the yeast URA3 gene to E. coli lacZ gene that encodes a β-galactosidase activity expressed in yeast (Rosbash & Broach, 1980). The resident 2 μm plasmid replication origin but contains a site for the site-specific recombinase encoded by the FLP gene product, which is encoded by the endogenous 2 μm circle plasmid (Braasch & Hicks, 1980; Braasch et al., 1982). Plasmid pBR73 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 (Braasch & Hicks, 1980).

As in the transformation with SacI-cleaved pCIH308 described above, lacZ’ transformants occurred frequently when pBR73 DNA, linearized by SacI cleavage in the lacZ’ gene, was added to DBY1226 (lacZ’ ) 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 SacI site (see Fig. 1) stimulated the yield of LacZ’ all of these lacZ’, again with plasmids.

The lacZ’ transformation was used with the approach above, alone did not yield lacZ’ with plasmids.

Transformants of these transfectant clones were further analyzed by electron microscopy (see Fig. 6 (see Fig. 6) of the 14 linearized plasmids, six symmetrically symmetrical junctions were observed, 8 pairs between the 2 μm base-pairs.

Apparent that the 2 μm circle plasmid DNA structures exist with a 2 μm circle plasmid DNA.

These 13 plasmids were sequenced by transforming DNA, provided no hybridization between two plasmids, neither could be detected in the hybrid plasmid transformation experiments.

All of the lacZ’ transformants have been observed to contain a 2 μm circle plasmid.

In summary, the SacI site has been found to be a derivative of a plasmid in a SacI site.

Figure 5. Electron microscopic analysis of inverted dimer plasmid products. (a) Micrograph of a heteroduplex containing a three-way duplex DNA joint. The putative inverted dimer plasmid "IV" (Fig. 2), purified from E. coli, was digested with SacI, which would be expected to cleave the plasmid at 2 symmetrical sites to generate 2 fragments of 60 kbp and 190 kbp (Fig. 3). These cleavage products were denatured and annealed with SacI-cleaved pCIH307, a derivative of pCIH308 (Fig. 1) that contains a 1-kbp insert of yeast CEN5 DNA (Maine et al., 1984) in its pBR322 BamHI 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 pCIH307 molecule (broken lines) and one strand of the 190-kbp fragment of the inverted dimer plasmid (continuous line). (c) Micrograph of stem-loop and duplex back products of a partial inverted dimer plasmid. The SacI cleavage products of a partial pBR73 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 I molecule (size standard); b, a stem-loop molecule with an approximately 8-kbp duplex stem and a 24-kbp single-strand loop; c, an approximately 4-kbp duplex DNA snap-back molecule. These structures are discussed further in the text.
nucleases gave pCH308 a 1 site and, in each case, the junction observed suggested variable two plasmids compatible with the junctions 0 fragment to yield two plasmids and 19-0 kb (Fig. 3). Sulf digested inverted dimer plasmid DNA was denatured and annealed with Sulf-digested pCH307, a derivative of pCH308 with a 1.5 kb insert of cloned yeast CEN5 DNA (Maine et al. 1984) in the pBR322 BamHI 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 hybrid structure is apparently a heteroduplex between both strands of a Sulf-linearized pCH307 molecule and one strand of the 19.0 kb Sulf 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 this structure shown in the Figure would indicate that the symmetrical center of the 19.0 kb Sulf fragment is near the position expected for the SacI site. The other structures detected that contained a threeway duplex DNA joint appeared to be a heteroduplex between both strands of a Sulf-linearized pCH307 molecule and one strand of the 40.0 kb Sulf fragment of an inverted dimer plasmid.

Four of the putative inverted plasmid fragments were also characterized by denaturation and self-annealing after digestion with Sulf (see Fig. 3). Each with plasmid DNA (plasmids I, IV, V and VI, Fig. 2), electron microscopy examination revealed two site 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 pBR73, 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). 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). Plasmid pBR73 transforms yeast with a high frequency and is maintained as an autonomously replicating plasmid, presumably by incorporating a complete 2 µm cellular plasmid via FLP-promoted recombination (Broach & Hicks, 1980).

As in the transformation with SacI-cleaved pCH308 described above, Leu2+ transformants occurred frequently when pBR73 DNA, linearized by SacI cleavage in the lacZ gene, was added to DSY1226 (leu2+) sporeplasts 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 SacI 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 SacI-cleaved pBR73 DNA alone did not, however, contain head-to-head dimers composed of the linear pBR73 molecules. Gel-transfer hybridization analysis of DNA from 14 of these transformants, and restriction and electron micrographic 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 200,000 base-pair inverted repeat (IR) sequences of 2 µm circle. Between these IR sequences is 284 kb of unique sequence. This is demonstrated by the formation of stem-loop molecules upon denaturing and self-annealing the SacI cleavage products of purified plasmid DNA (Fig. 5). The two predominant structures detected by electron microscopy are duplex molecules of length of half the length of the 8.5 kb Sulf fragment, which potently contains the symmetrical junction, and stem-loop molecules with an 8.5 kb duplex stem and a single-strand loop of approximately 2.9 kb. These observations and restrictions analysis of these plasmids indicate that the 2.9 kb unique sequence is the large (2774 base-pairs) unique region of the 2 µm circle plasmid (Hartley & Donelson, 1984) which contains the 2 µm circle replication origin (Broach & Hicks, 1980), a DNA segment that is not present in pBR73. 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 pBR73 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 pBR73 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 pBR73 cleaved at sites other than the SacI site in the lacZ gene. These were observed with BamHI-digested pBR30 (Paico et al., 1982), and with BamHI-digested pBR30 (Broach & Hicks, 1980), cleavages which create plasmid ends in the pBR322 sequence. When pBR73 (Fig. 1) was digested with either BamHI, or both BamHI and SacI, the lacZ+ 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 SclI-cleaved pR373 and a possible mechanism for its formation. Structure I, the head-to-head dimer plasmid that could be formed from the pR373 linear molecule (L), contains two sites for FLP-mediated site-specific recombination (arrows indicate the positions of the 2 μm plasmid 59 base-pair inverted repeat (IR) sequences, which contain the site of FLP-mediated recombination; Brosch 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 recombinant event). The proficiency of this recombination system makes this event likely, but it also appears to be essential for replication of a plasmid bearing (structure III) would be expected to be unstable because it contains 2 FLP recombinase 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. DL loss is therefore not surprising. An open arrow (Ⅲ) indicates the position of Scl restriction sites.

pR373 DNA cleaved with RedI, which generates plasmid DNA ends in the lacZ and lacA genes.

(d) Dependence of inverted dimer formation on the presence of 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 SclI-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 lacZ+ 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+ transformants examined by gel-transfer hybridization analysis were found to harbor monomer plasmids with a deletion encompassing the Scl 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 & Strook (1983) and Suzuki et al. (1983).

There appear to be two separable activities of carrier DNA in transformation. With the presence of phage φX174 RF1 DNA as carrier, the yield of transformants with SclI-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 Scl site. When the φX174 RF1 carrier DNA has been linearized by PstI cleavage and then sonicated, the yield of transformants is more substantially increased than with intact φX174 RF1 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 X DNA cleaved with SalI (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 pR3308 described earlier, fragmented dimer plasmids were detected in 3% of the transformants. In the experiment with linearized plasmid pR373, 5% of the transformants contained "partial" inverted dimer plasmids, apparent derivatives of inverted dimer plasmids formed via FLP-mediated specific recombination (Fig. 6). The presence of sonicated carrier DNA during transformation appears to account for the difference between this result and the previously reported formation of monomer products. We find that in the absence of carrier DNA, 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 lines 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 & Strook, 1983), and plasmids formed by the joining of 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 relatively poor joining of restriction fragments.

This would be expected 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 recircularization would be favored. Furthermore, the formation of inverted dimer plasmids is not a special property of linear molecules bearing SclI-generated lacZ ends. Transformation with linearized plasmid bearing BstI-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 SclI-linearized pR373, 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
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 pCH08 described in Table 1, inverted dimer plasmids were detected in 82% of the transformants. In the experiment with linearized plasmid pBR73, 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 linear segments 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. In the absence of repair mechanisms such as recombination (Ort-Weaver & Stoszek, 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 lnZ 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 SacI-generated lnZ ends. Transformation with linearized plasmids bearing BstEII-generated ends in the lnZ and SacI genes, or with ends within the pBR322 or URA3 gene sequences, in an outcome similar to that with SacI-linearized pBR73; 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 enzyme 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 respiratory-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 (Locke 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, 1938). Genetic experiments may have revealed such a process in yeast (Haber et al., 1984). The formation of head-to-head 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. Alternatively, each free end of a linear duplex, 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 homologous 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 λ, pTX174 RFI DNA) do not promote the formation of inverted dimers, small DNA fragments (phage λ DNA cleaved with RalI) 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 (pCH08). 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

4. Discussion

In the experiments reported here, the predominant plasmid product of transformation
a high concentration of free DNA ends marks 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 SceI 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 (Rennick, 1976) and the high frequency of plasmid, chromosome recombination observed with a plasmid cleaved in a region of chromosomal homology (Or-Waver et al., 1981; Or-Waver & Stotzak, 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 inverteddimers. 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.

We thank Michael Lichter, Mark Rose, Karen Overby and Connie Holm for their advice and ideas and for providing plasmid DNA.

References


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.

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50% of the transformants, depending on the peculiar 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 N.Z 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 linearized substrates necessary for inverted dimer formation, and yield a monomeric 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.

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