Synapsis-Mediated Fusion of Free DNA Ends Forms Inverted Dimer Plasmids in Yeast

Sam Kunes, David Botstein[1] and Maurice S. Fox

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Manuscript received March 15, 1989
Accepted for publication September 22, 1989

ABSTRACT

When yeast (*Saccharomyces cerevisiae*) is transformed with linearized plasmid DNA and the ends of the plasmid do not share homology with the yeast genome, circular inverted (head-to-head) dimer plasmids are the principal product of repair. By measurements of the DNA concentration dependence of transformation with a linearized plasmid, and by transformation with mixtures of genetically marked plasmids, we show that two plasmid molecules are required to form an inverted dimer plasmid. Several observations suggest that homologous pairing accounts for the head-to-head joining of the two plasmid molecules. First, an enhanced frequency of homologous recombination is detected when genetically marked plasmids undergo end-to-end fusion. Second, when a plasmid is linearized within an inverted repeat, such that its ends could undergo head-to-tail homologous pairing, it is repaired by intramolecular head-to-tail joining. Last, in the joining of homologous linearized plasmids of different length, a shorter molecule can acquire a longer plasmid end by homologous recombination. The formation of inverted dimer plasmids may be related to some forms of chromosomal rearrangement. These might include the fusion of broken sister chromatids in the bridge-breakage-fusion cycle and the head-to-head duplication of genomic DNA at the sites of gene amplifications.

THE molecular consequences of chromosomal breakage have long been in question. Possible outcomes include recombination and rearrangement, processes whose mechanistic relationship is unclear. A central feature of the processing of broken chromosomal ends may be the reactive properties of free DNA ends. Such ends might fuse together randomly, or serve as substrates for homologous, or illegitimate recombination. Homologous recombination provides an avenue by which to restore a broken chromosome to the wild-type structure. The idea that free DNA ends can serve as initiators in homologous recombination has received strong experimental support (reviewed in THALER and STAHL 1988). Rearrangement might occur when free DNA ends initiate recombination at ectopic homologous or nonhomologous sites. Rearrangement might also result from DNA end-to-end joining. To what extent, then, do the reactions underlying homologous recombination play a role in rearrangement mechanisms?

One approach to elucidating the in vivo processing of free DNA ends is by DNA-mediated transformation of yeast with linearized plasmid DNA (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981). A plasmid broken by restriction nuclease cleavage in a region of homology with the yeast genome recombines efficiently at its cognate chromosomal locus. The product is, with equal likelihood, a chromosomally-integrated plasmid or an extrachromosomal circular plasmid (ORR-WEAVER and SZOSTAK 1983). Free DNA ends can, on the other hand, join end-to-end in an apparently nonhomologous reaction (ORR-WEAVER and SZOSTAK 1983; SUZUKI *et al.* 1983). Such end-to-end joining products are recovered exclusively on transformation with a plasmid linearized in region lacking homology with the yeast genome (KUNES, BOTSTEIN and FOX 1985). A third pathway for processing free DNA ends in yeast is observed when, in the absence of chromosomal homology, a linearized plasmid is introduced along with an excess of highly fragmented nonhomologous carrier DNA (KUNES, BOTSTEIN and FOX 1985). The presence of carrier DNA results in a new population of transformants, most of which harbor a circular inverted (head-to-head) dimer of the original linearized plasmid. The role of fragmented carrier DNA in forming these products is not clear. It could be to induce an additional DNA repair pathway, or to protect a plasmid repair intermediate that would otherwise be destroyed by cellular nucleases.

An inverted dimer plasmid could be formed from one plasmid molecule that is replicated after its entry into the cell. Such a mechanism might account for the head-to-head union of the daughter DNA duplexes. For example, joining of the 5′ and 3′ DNA strands at each duplex plasmid end would form a hairpin structure that, on replication, could yield an inverted dimer

[1] Present address: Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080.

The publication costs of this article were partly defrayed by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

plasmid. If, on the other hand, the joining reaction utilizes two linearized plasmids freely-existing in the cell, there must be some mechanism for first aligning them. Perhaps the plasmid molecules interact first by homologous pairing, after which the ends in close proximity, the homologous ends, are joined.

We have performed experiments aimed at distinguishing between these possible mechanisms. By transforming yeast with mixtures of genetically marked plasmids, and by studying the DNA concentration dependence of transformation with linearized plasmid DNA, we show that two plasmid molecules are required to form an inverted dimer plasmid. Secondly, we report several observations which suggest a role for homologous pairing in the reaction. These observations provide evidence for a novel role of homologous pairing in a rearrangement process.

MATERIALS AND METHODS

Strains and media: Yeast strains DBY1226 (*MAT***a** *his4-519 met8-1 leu2-3,112 ura3-3*) and DBY747 (*MAT***a** *his3-Δ1 trp1-289 ura3-52 leu2-3,112*) were constructed in this laboratory by standard methods (SHERMAN, FINK and LAWRENCE 1979). To construct DBY2523, the *leu2-3,112* locus of DBY747 was replaced by the method of ROTHSTEIN (1983) with a *leu2::HIS3* substitution allele constructed *in vitro*. The substitution replaces the internal *LEU2 Cla*I to *Eco*RV sequence (ANDREADIS *et al.* 1982) with the *Bam*HI, *Xho*I fragment of *HIS3* (STRUHL 1985).

Yeast was grown in YEPD (complete) or SD (minimal) medium (SHERMAN, FINK and LAWRENCE 1979). *Escherichia coli* was grown in LB (complete) medium (MILLER 1972) containing, when appropriate, 100 μg ampicillin/ml (Sigma Chemical Co., St Louis, MO).

Plasmids: Plasmid pCH308 (Figure 1) was obtained from C. HOLM (Harvard University) and has been described (KUNES, BOTSTEIN and FOX 1985). A *ura3* derivative of pCH308, pSK136, was constructed by recircularizing *Nco*I-cleaved pCH308 DNA after filling in the *Nco*I-generated ends with *E. coli* DNA polymerase I. A *leu2* derivative of pCH308, pSK142, was constructed by recircularizing *Kpn*I cleaved pCH308 DNA which had been treated with T4 DNA polymerase to remove the single-stranded ends. A derivative of pCH308 containing the yeast *TRP1* gene, pSK301 (Figure 1), was constructed by inserting a *TRP1*-containing *Hin*dIII fragment derived from plasmid YRp7 (TSCHUMPER and CARBON 1979) into the pCH308 *Hin*dIII site between *ARS1* and *URA3*. A *trp1* derivative of pSK301 was constructed by filling in at the *TRP1 Hin*dIII site, yielding a 4-bp insertion mutation. Derivatives of pSK301 with *ura3* or *leu2* mutations were constructed as described above for pCH308. The resulting genetically marked plasmids are described in Figure 1.

Plasmids pSK225 and pSK227 (Figure 4) are derivatives of pSK136 and pSK142, respectively, constructed by replacing the *CYC1'lacZ Sal*I, *Nco*I fragment with a 12-kb *Sal*I-*Nco*I fragment containing a *lacZYA* head-to-head duplication, derived from pSK150, an inverted dimer plasmid formed after transformation with *Bam*HI-cleaved pRB73 (KUNES, BOTSTEIN and FOX 1985).

Plasmid pSK218 (Figure 8) is a derivative of the *ura3-3 LEU2* plasmid, pRB30 (FALCO *et al.* 1982) in which a 3' deleted *ura3* gene derived from pRB82 (ROSE and BOTSTEIN 1983) was inserted at the pRB30 *Bam*HI site.

The recombinant DNA methods employed in these constructions were performed essentially as described by MANIATIS, FRITSCH and SAMBROOK (1982).

DNA preparation and restriction enzyme cleavage: Plasmid DNA was isolated from *E. coli* by a modification (RAMBACH and HOGNESS 1977) of the method of CLEWELL and HELINSKI (1969). Plasmid DNA was further purified by banding once in a CsCl/ethidium bromide density gradient (RADLOFF, BAUER and VINOGRAD 1967). Supercoiled plasmid DNA was stored in TE buffer [10 mM-Tris (pH 8.0), 1 mM Na_2EDTA] at 4° or −20°. Yeast DNA was isolated by the method of HOLM *et al.* (1986) from cultures grown in liquid SD medium with selection for plasmid maintenance. Chicken erythrocyte DNA was obtained from Calbiochem-Behring (La Jolla, CA). After dissolution in TE, it was sonicated to an average molecular weight of 5×10^5, extracted three times with phenol/chloroform (1:1, v/v, equilibrated to pH 8.0), precipitated in ethanol three times and stored in TE buffer at 4°.

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

Yeast transformation: Yeast transformation by the spheroplasting method was performed essentially as described by HINNEN, HICKS and FINK (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 glusulase (Dupont Pharmaceuticals, Wilmington, DE). The final suspension contained spheroplasts at a concentration of 1×10^9/ml, of which typically 10% were colony-forming units in regeneration agar with complete supplements. In an individual transformation, 100 μl of spheroplast suspension was added to 10 μl of TE buffer containing plasmid DNA and 10 μg sonicated chicken erythrocyte (carrier) DNA, prepared as described above. Plating in regeneration agar on SD selective media was followed by incubation at 30° for 4–5 days. Selection for Leu^+, Trp^+, or Ura^+ transformants was made in the presence of a growth-limiting quantity of leucine (1 μg/ml), tryptophan (0.4 μg/ml) or uracil (0.1 μg/ml), respectively. With the use of these conditions, transformation yields with either closed circular or linearized plasmid DNA are substantially increased, presumably by allowing time for plasmid gene expression prior to cell starvation. Furthermore, under these conditions, transformation with a plasmid containing the *URA3*, *LEU2* and *TRP1* genes (pSK301, Figure 1) results in approximately equal yields on single selection for Ura^+, Leu^+, or Trp^+ transformants. The recovery of inverted dimer plasmids does not require the provision of these nutrients.

Alkaline DNA gel-transfer hybridization analysis: The end-to-end junction fragments of inverted dimer plasmids were best visualized by a modification of the method of SOUTHERN (1975) in which DNA transfer to a filter was carried out under denaturing conditions. Yeast DNA isolated from a plasmid-carrying strain was digested with restriction endonuclease and electrophoresed in an agarose gel. The DNA was denatured *in situ* by washing for 30 min with agitation in 0.5 M NaOH 1.0 M NaCl. The DNA was transferred to a Gene Screen-Plus filter membrane (New England Nuclear Co., Boston, MA) by capillary elution with 0.5 M NaOH 1.0 M NaCl. The filter was subsequently washed briefly in 0.5 M NaOH, rinsed with distilled water, and neutralized by washing briefly in 0.2 M Tris (pH 7.5) 2 × SSC. Hybridization was performed as recommended by the manufacturer.

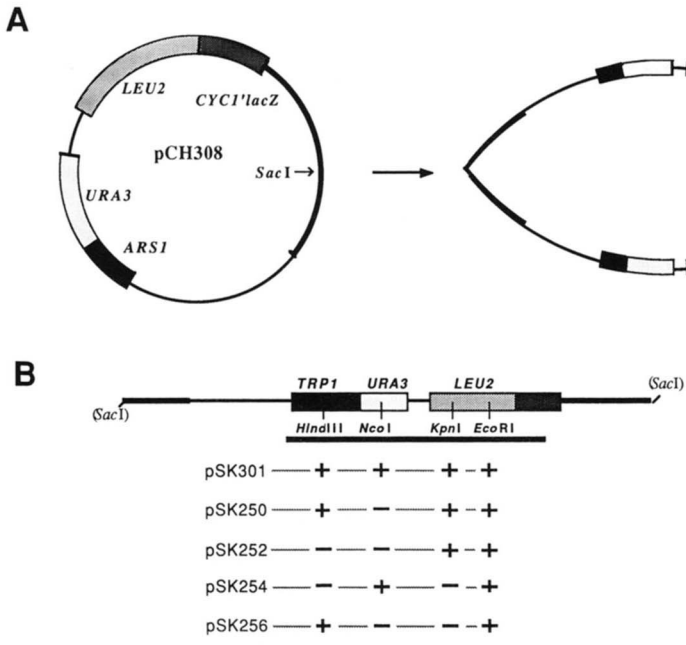

FIGURE 1.—Formation of inverted dimer plasmids after transformation of yeast with linearized plasmid DNA: A, Plasmid pCH308 (left of arrow; see KUNES, BOTSTEIN and FOX 1985, for structural details) and the circular inverted (head-to-head) dimer plasmid (right of arrow) resulting on transformation in the presence of sonicated carrier DNA with *Sac*I-linearized pCH308 DNA. The product is not a complete dimer of the original plasmid; deletions, typically of 100 to 300 basepairs, are present at the novel end-to-end junctions (KUNES, BOTSTEIN and FOX 1985). Thus, *Sac*I sites are not regenerated by the joining events. B, Structure of genetically marked plasmids containing the yeast *TRP1*, *URA3* and *LEU2* genes. Plasmid pSK301, shown in *Sac*I-linearized form, is a derivative of pCH308 containing the yeast *TRP1* gene (TSCHUMPER and CARBON 1979). Derivatives of pSK301 with *in vitro* generated mutations in the plasmid's *TRP1*, *URA3* or *LEU2* genes were constructed by destroying the indicated restriction sites, and thus generating frameshaft mutations. The resulting family of marked plasmids is shown.

RESULTS

To determine the number of plasmid molecules required for the formation of an inverted dimer plasmid, we investigated the DNA concentration dependence of transformation with linearized plasmid DNA. If the formation of an inverted dimer plasmid requires only a single plasmid molecule, this dependence should be first order. A requirement for two molecules would be reflected by a second order concentration dependence. A second approach was to transform yeast with a mixture of homologous plasmids which were genetically marked. A bimolecular mechanism would generate heterozygous dimer plasmids.

A set of genetically marked plasmids is shown in Figure 1B. Plasmid pSK301 contains the cloned yeast genes *TRP1*, *URA3* and *LEU2*, a *CYC1'lacZ* gene fusion, and the *ARS1* element, which confers autonomous plasmid replication in yeast. Genetically marked derivatives of pSK301 were constructed by *in vitro* modifications at appropriate restriction sites (see MATERIALS AND METHODS) to generate the set of *trp1*, *ura3* and *leu2* derivatives shown. These plasmids can be linearized by *Sac*I cleavage in their resident *lacZ* gene, generating plasmid molecular ends lacking homology with the yeast genome.

DNA concentration dependence of transformation with a linearized plasmid: The DNA concentration dependence of transformation was determined by transforming yeast strain DBY747 (*trp1 leu2 ura3*) with various amounts of an equimolar mixture of pSK250 (*TRP1 ura3 LEU2*) and pSK254 (*trp1 URA3 leu2*). In the experiment shown in Figure 2, with the plasmid DNA mixture in closed circular form, single selection for Leu^+ transformants (and also for Ura^+ transformants, not shown) reveals a first order concentration dependence, while double selection for Ura^+ Leu^+ transformants results in a second order dependence. These outcomes are expected on the basis of a unimolecular requirement for the transformation event. On the other hand, with the plasmid DNA mixture linearized by *Sac*I cleavage, both single and double selections result in an approximately second order concentration dependence over a broad range of DNA concentrations. This observation was reproduced in several similar experiments with these plasmids or with the closely related plasmids, pSK136 and pSK142 (shown in Figure 6A). As will be seen below, the recovery of Ura^+ Leu^+ transformants is due to the formation of heterozygous inverted dimer plasmids. The recovery of transformants with a second order concentration dependence on both single and double selections suggests that two plasmid molecules participate in the repair of a linearized plasmid.

At very low DNA concentrations, however, Leu^+ transformants occur more frequently than would be expected for a bimolecular event (Figure 2). All of 12 Leu^+ transformants from this region of the concentration curve were found to harbor either a monomer plasmid of the parental structure or a monomer plasmid with deletion including the *Sac*I site. It is possible that the recovery of these products is due to low levels of circular plasmid DNA contaminating the linearized plasmid DNA preparation. On the other hand, the reclosure of linearized plasmids without deletion for-

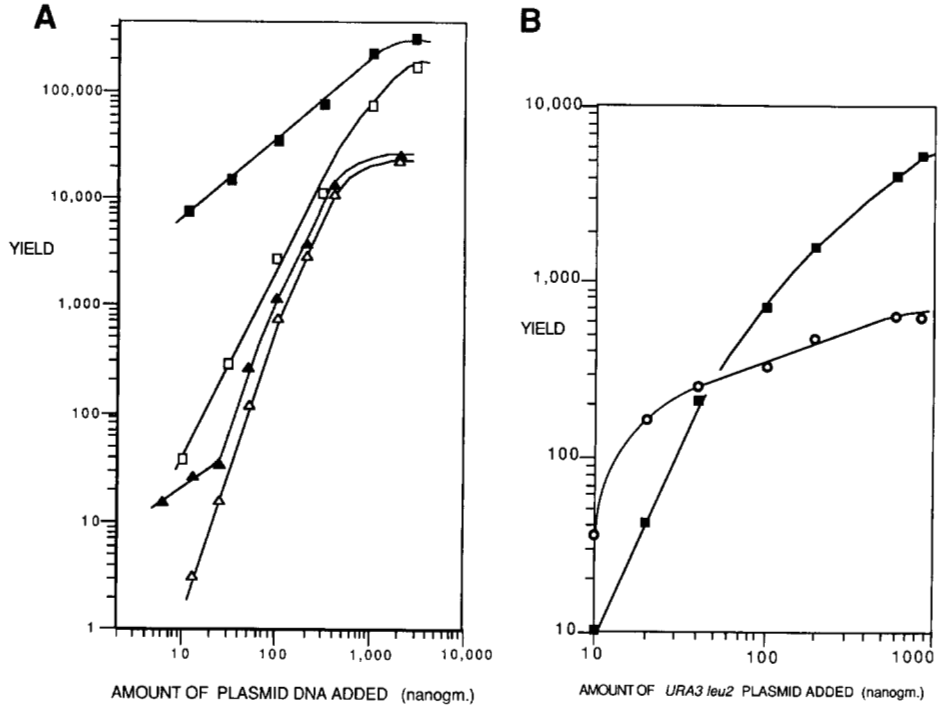

FIGURE 2.—DNA concentration dependence of transformation with linearized plasmid DNA: A, Transformation with an equimolar mixture of two genetically marked plasmids. Transformation of yeast strain DBY747 (*trp1 ura3 leu2*) was performed with various amounts of a 1:1 molar mixture of pSK250 (*TRP1 ura3 LEU2*) and pSK254 (*trp1 URA3 leu2*), depicted in Figure 1B. The plasmid mixture was either linearized by *Sac*I cleavage or in closed circular form. The log-log plot shown gives the total yield of transformants on either single (Leu^+) or double (Ura^+ Leu^+) selection. The curves are denoted as follows: ■, Leu^+, closed circular DNA; □, Ura^+ Leu^+, closed circular DNA; ▲, Leu^+, *Sac*I-cleaved DNA; △, Ura^+ Leu^+, *Sac*I-cleaved DNA. B, Dependence of transformation with a linearized *ura3 LEU2* plasmid on the presence of a linearized *URA3 leu2* plasmid. Two genetically marked derivatives of pCH308 (Figure 1A) were constructed as described in MATERIALS AND METHODS. *Sac*I-linearized DNA mixtures containing 20 ng of the *ura3 LEU2* plasmid, pSK136, and increasing amounts of the *URA3 leu2* plasmid, pSK142, were used to transform DBY1226 (*leu2 ura3*). The log-log plot shown gives the total yield of transformants with either Ura^+ (■) or Leu^+ (○) single selection as the amount of the *URA3 leu2* plasmid is increased.

mation has been reported in yeast (ORR-WEAVER and SZOSTAK, 1983). Secondly, nucleotide sequence analysis of the novel junctions of the deletion-bearing circular products reveals a characteristic structure shared with the end-to-end junctions of inverted dimer plasmids (our unpublished results). Hence we suggest that these products form in yeast from linearized plasmid DNA. There would thus be two repair pathways for linearized plasmid DNA in the absence of a target for homologous recombination; a bimolecular reaction that generates inverted dimer plasmids and a much less efficient unimolecular head-to-tail joining reaction.

A physical analysis was employed to show that the recovery of Ura^+ Leu^+ transformants with the linearized plasmid mixture is due to the formation of heterozygous inverted dimer plasmids. As shown in Figure 3, each dimer could be expected to bear the genotype *TRP1/trp1*, *ura3/URA3*, and *LEU2/leu2*, with the markers positioned according to their original plasmid linkage configuration. Since the mutant alleles result from the destruction of restriction sites, each plasmid's genotype could be physically determined. Of 32 plasmids analyzed by a combination of restriction analysis after rescue of the plasmids in *E. coli* (Figure 3), and gel-transfer hybridization analysis of DNA from the yeast transformants (not shown), 28 were indeed heterozygous, with the markers distributed in the expected pattern. However, one plasmid was an *URA3* homozygote, and three plasmids were *trp1* homozygotes. Consistent with the latter observation, 8% of the transformants obtained with Ura^+ Leu^+ double selection were found to be Trp^- and to contain homozygous *trp1* inverted dimer plasmids. On the other hand, when a heterozygous *TRP1/trp1* inverted dimer plasmid, purified from *E. coli*, was reintroduced to yeast in closed circular form, less than 1% of the resulting Ura^+ transformants were Trp^-. These observations suggest that the linearized plasmid molecules that undergo end-to-end joining experience an enhanced level of homologous recombination. To extend these observations, we are studying recombination between heteroallelic *leu2* plasmids. Preliminary results suggest that the frequency of such homologous recombination events increases as the plasmid markers are positioned more and more closely to a free DNA end.

Further evidence that the formation of inverted dimer plasmids is a bimolecular reaction was obtained by varying the concentration of only one of two linearized plasmids in a DNA mixture. Transformation was carried out with mixtures containing a constant

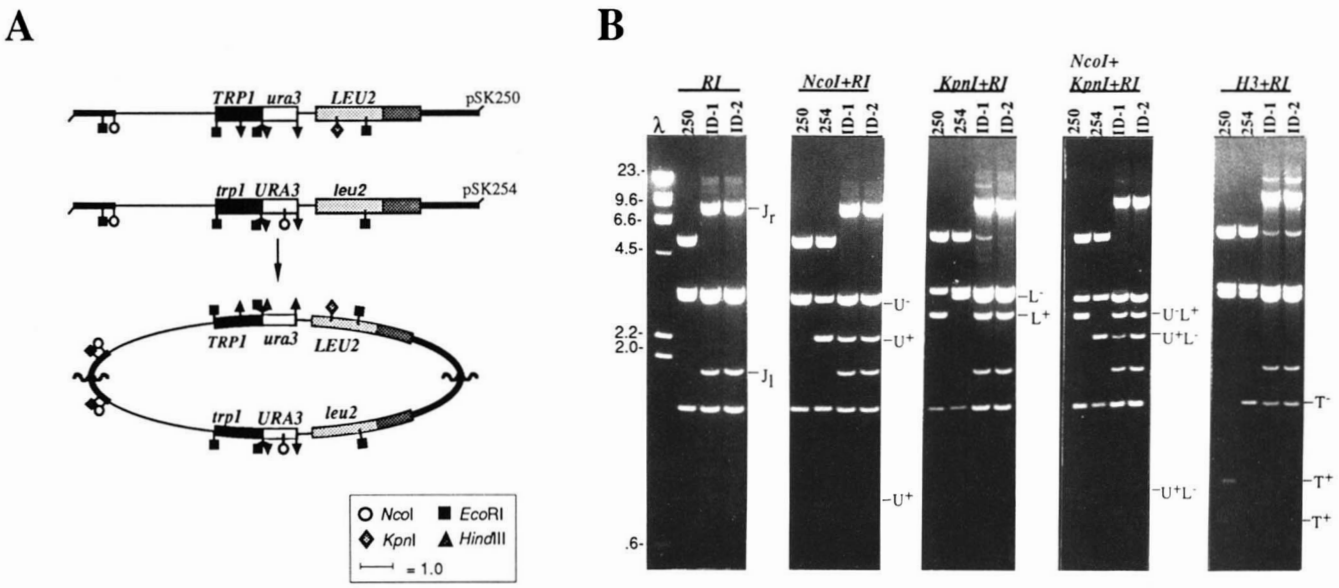

FIGURE 3.—Formation of heterozygous inverted dimer plasmids after transformation with genetically marked plasmid DNA. A, Plasmids pSK250 (*TRP1 ura3 LEU2*) and pSK254 (*trp1 URA3 leu2*) are shown in *Sac*I-linearized form. Shown beneath the arrow is the heterozygous inverted dimer plasmid expected as result of the fusion of pSK250 and pSK254. The restriction map of the dimer product indicates the expected positions of restriction sites destroyed in the process of generating the *trp1*, *ura3* and *leu2* markers (as shown in Figure 1B). B, A restriction analysis of two heterozygous inverted dimer plasmids, ID1 and ID2, isolated after transfer from yeast to *E. coli*. Phage λ DNA cleaved with *Hin*dIII, and pSK254 and pSK250 DNA, cleaved as indicated, are included as controls. The restriction cleavages are chosen to assay for the presence of restriction site mutations introduced as genetic markers in the plasmid's *TRP1*, *URA3* and *LEU2* genes. The *Eco*RI-generated head-to-head and tail-to-tail junction fragments are denoted J_l and J_r. The restriction fragments corresponding to a particular genotype are indicated as follows: U^+, *URA3*; U^-, *ura3*; L^+, *LEU2*; L^-, *leu2*; T^+, *TRP1*; T^-, *trp1*. The two inverted dimer plasmids possess restriction fragments consistent with the presence of both the wild-type and mutant alleles for each of the three genes.

low concentration of the *ura3 LEU2* plasmid, pSK136 (see Figure 1), and increasing amounts of the *URA3 leu2* plasmid, pSK142. With the addition of *Sac*I-linearized plasmid mixtures, the frequency of Leu^+ transformants increased with the presence of increasing concentrations of the *URA3 leu2* plasmid (Figure 2). The frequency of Leu^+ transformants increased initially at a high rate, but with greater amounts of the linearized *URA3 leu2* plasmid, the increase was less substantial. Hence the *URA3 leu2* molecules appear to act as partners with *ura3 LEU2* molecules in the formation of inverted dimer plasmids.

Proportion of heterozygous inverted dimers after transformation with genetically marked plasmids: To determine whether heterozygous inverted dimer plasmids are recovered in the proportion expected for a bimolecular reaction, transformation was performed with a 1:1:1 molar mixture of pSK252 (*TRP1 ura3 leu2*), pSK254 (*trp1 URA3 leu2*), and pSK256 (*trp1 ura2 LEU2*). For a bimolecular reaction, transformants are expected bearing any of six possible phenotypes. Pairwise combination of the three marked plasmids would give rise to transformants bearing any two of the three wild-type markers, *TRP1*, *URA3*, and *LEU2*, as well as transformants resulting from homozygous pairings that would display only one of the wild-type markers. On the other hand, were inverted dimer plasmids formed from a single plasmid molecule, a transformant would display only one of the three plasmid genotypes. Trp^+ Ura^+ Leu^+ transformants could be expected if more than two molecules often contribute markers to an inverted dimer plasmid.

Yeast strain DBY747 (*trp1 ura3 leu2*) was transformed with 50 ng and 200 ng of the *Sac*I-linearized plasmid mixture. Both quantities are significantly below DNA saturation (see, for example, Figure 2), which insures that the dimer plasmids recovered are formed from the minimum possible number of participating molecules. Transformants obtained with either Trp^+, Ura^+, or Leu^+ single selection were tested for presence of the remaining two wild-type markers (Table 1). At the lower DNA concentration, transformants displaying two of the three wild-type markers account for 50% to 62% of the total. At the higher DNA concentration, a slightly greater fraction, 60% to 70%, displayed any two of the three wild-type markers. At either DNA concentration, transformants bearing all three wild-type markers were comparatively rare. This outcome argues strongly against the frequent participation of more than two molecules in the formation of an inverted dimer plasmid, at least at subsaturating DNA concentrations. However, in the case of the random assortment of the molecules that participate in a bimolecular reaction, we expect that 80% of the transformants would display a second

TABLE 1

Proportion of heterozygous inverted dimer plasmids resulting with a mixture of genetically marked linearized plasmids

DNA	Primary selection	Unselected wild-type marker					
		Ura$^+$	Leu$^+$	Trp$^+$	(++)	Total	% het
50 ng DNA mix	Ura$^+$	—	45	45	2	146	62
	Leu$^+$	39	—	50	2	148	60
	Trp$^+$	25	37	—	0	125	50
200 ng DNA mix	Ura$^+$	—	46	29	5	103	73
	Leu$^+$	27	—	38	2	104	62
	Trp$^+$	23	43	—	3	94	70

Yeast strain DBY747 (*trp1 ura3 leu2*) was transformed with either 50 ng or 200 ng of a 1:1:1 mixture of *Sac*I-linearized pSK252 (*trp1 ura3 LEU2*), pSK254 (*trp1 URA3 leu2*), and pSK256 (*TRP1 ura3 leu2*). Transformants obtained with primary selection for Trp$^+$, Ura$^+$, or Leu$^+$ were then tested for the presence of the remaining two wild-type markers. For each single selection, the table gives the number of transformants displaying one or both (++) of the remaining wild-type markers, and the percent of the total (% het) which appear to harbor heterozygous inverted dimer plasmids.

wild-type marker. This discrepancy is accounted for by two factors. First, about one-half (15/33) of the transformants at the lower DNA concentration that had displayed a single wild-type marker were found to harbor a monomer plasmid; the remainder contained an inverted dimer plasmid. Secondly, as indicated above, recombination events between heteroallelic molecules can result in the loss of a wild-type marker.

Intramolecular repair of a linearized plasmid with homologous but inverted ends: One possible mechanism that could account for the prominence of head-to-head plasmid joining is that the two plasmid molecules are first aligned by homologous pairing. Homologous pairing might facilitate joining by bringing two DNA ends into close proximity. If this was the case, an intramolecular head-to-tail joining reaction might repair a plasmid linearized within a head-to-head duplication (Figure 4). This molecule would bear ends that are homologous but inverted, permitting head-to-tail homologous pairing. The resulting intramolecular joining reaction should generate a monomeric circular product (Figure 4).

To test this idea, the *lacZ* region of the pCH308 derivatives, pSK136 (*ura LEU2*) and pSK142 (*URA3 leu2*) was replaced with a 12-kb fragment containing two inverted copies of the *E. coli lacZYA* region (Figure 4). The resulting plasmids, pSK225 (*ura3 LEU2*) and pSK227 (*URA3 leu2*), are cleaved twice at symmetrical positions by *Sac*I, yielding linear molecules with homologous *lacZ* ends. As shown in Figure 4, on transformation with a *Sac*I-cleaved equimolar mixture of pSK225 and pSK227, single selection for Ura$^+$ transformants revealed a first-order DNA concentration dependence, consistent with repair in a unimolecular reaction. On the other hand, the recovery of Ura$^+$ Leu$^+$ transformants displays the second order concentration dependence expected on the basis of the independent incorporation of single linear molecules. This outcome was reproduced in a second independent experiment (data not shown). Relatively few of the transformants obtained with either Leu$^+$ or Ura$^+$ single selection displayed the Ura$^+$ Leu$^+$ phenotype, unlike the outcome with the original plasmids, pSK136 and pSK142 (Table 2). Hence, with the plasmids bearing head-to-tail homology, heterozygous products are indeed rare at low DNA concentrations, consistent with repair in a unimolecular event. Finally, only one of 24 Leu$^+$ transformants examined by DNA gel-transfer hybridization (Figure 5) displayed plasmid restriction fragments consistent with the presence of a head-to-head dimer plasmid; the remaining 23 contained the monomer product expected from a head-to-tail joining reaction. It is worth noting, however, that the dimer product expected from the bimolecular reaction (shown in Figure 4) could, by intramolecular recombination between the duplicated *lacZYA* elements, resolve to generate the same monomeric product as expected from the unimolecular event. This possibility is not consistent with recovery of these transformants with a unimolecular DNA concentration requirement.

Inverted dimer plasmids from joining linearized plasmids of different lengths: When a plasmid used in yeast transformation is cleaved at two sites within a resident yeast sequence, such that a DNA segment is excised, homologous recombination with the genomic locus restores the excised material, an outcome termed gap repair (ORR-WEAVER, ROTHSTEIN and SZOSTAK 1981). This suggests the possibility that the end-to-end fusion of two homologous linearized plasmids, one of which has been made shorter than the other at an end, might be accompanied by the recovery of the material absent from the shorter plasmid end. This outcome would be consistent with the suggested role of homologous pairing in the joining process.

This question was addressed by assaying for the joining of *Sac*I-linearized pSK142 (*URA3 leu2*) with the 1.3-kb shorter *Nco*I, *Sac*I-generated molecule of pSK136 (*ura3 LEU2*) shown in Figure 6. An equimolar mixture of these two plasmid DNAs was introduced to DBY1226 at various total DNA concentrations. Single selection for Leu$^+$ or Ura$^+$ transformants resulted in transformants with a second order concentration dependence, as expected for a bimolecular reaction. As shown in the Table 3, the fraction of transformants which display the Ura$^+$ Leu$^+$ phenotype is comparable to that observed with both linearized plasmids of equal length, produced by *Sac*I cleavage alone (see Table 2). Hence, the presence of the short-

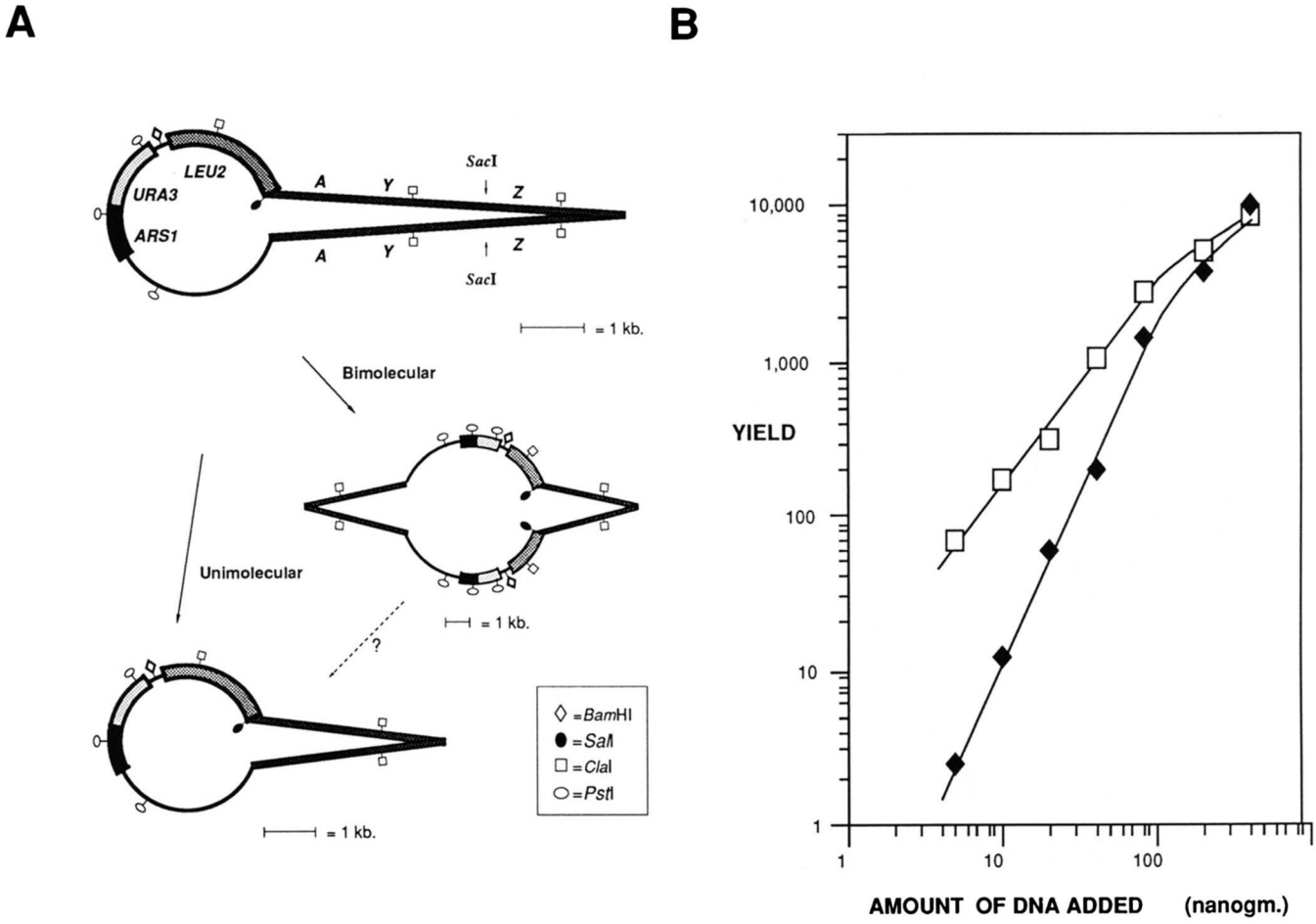

FIGURE 4.—Transformation with a plasmid DNA cleaved within a head-to-head duplication. A, Plasmids pSK255 (*ura3 LEU2*) and pSK227 (*URA3 leu2*) are derivatives of pCH308 (Figure 1) in which the original *CYCl'lacZ* gene fusion is replaced by two head-to-head copies of the *E. coli lacZYA* region, forming a 12-kb inverted duplication. The *lacZ*, *lacZ* junction is asymmetric, allowing these plasmids to propogate in *E. coli*. The monomer product shown at the bottom is expected as a result of intramolecular (head-to-tail) joining following transformation with *Sac*I-cleaved pSK225 and pSK227. The possible bimolecular head-to-head joining product is shown on the right. B, Transformation of yeast strain DBY747 (*trp1 ura3 leu2*) was performed with various amounts of a *Sac*I-cleaved equimolar mixture of the plasmids containing the *lacZYA* inverted duplication, pSK225 (*ura3 LEU2*) and pSK227 (*URA3 leu2*). The log-log plot gives the total yield of transformants as the amount of the DNA mixture was increased. □, Leu^+ selection; ♦, Ura^+ Leu^+ double selection.

TABLE 2

Proportion of heterozygous products resulting with plasmids linearized between two head-to-head copies of *lacZYA*

Plasmids	Primary selection	Fraction Ura^+ Leu^+/total with various amounts of DNA (ng)			
		10	25	50	100
lacZYA	Ura^+	5/19	15/60	10/28	40/86
	Leu^+	8/36	32/101	22/44	26/54
lacAYZ'ZYA	Ura^+	1/45	2/60	4/54	5/59
	Leu^+	4/156	4/75	5/60	

Yeast strain DBY1226 (*ura3 leu2*) was transformed with a *Sac*I-cleaved equimolar mixture of pSK136 (*ura3 LEU2*) and pSK142 (*URA3 leu2*) or a *Sac*I-cleaved equimolar mixture of the plasmids harboring a *lacZYA* inverted repeat (*lacAYZ'ZYA*), pSK225 (*ura3 LEU2*) and pSK227 (*URA3 leu2*), shown in Figure 4. Ura^+ or Leu^+ transformants were tested for the Ura^+ Leu^+ phenotype. The table gives the fraction of those tested that were found to be Ura^+ Leu^+.

ened end does not seem to interfere with joining a longer molecule.

To determine the structure of the end-to-end junctions of these inverted dimer plasmids, DNA from Ura^+ Leu^+ transformants was cleaved with *Eco*RI and visualized by gel-transfer hybridization analysis. As shown in Figure 6, the 1.3-kb segment removed in shortening the pSK136 molecule contains an *Eco*RI site. We thus determined whether this site had been restored. Of 21 Ura^+ Leu^+ inverted dimer plasmids examined (Figure 7), 10 did indeed bear an *Eco*RI-generated junction fragment consistent with the restoration of most or all of the 1.3-kb segment prior to joining with the longer pSK142 end. On the other hand, 11 of the 21 plasmids lacked both *Eco*RI sites, and bore an *Eco*RI-generated junction fragment consistent with the absence of most or all of extra material of the longer pSK142 molecule prior to fusion with

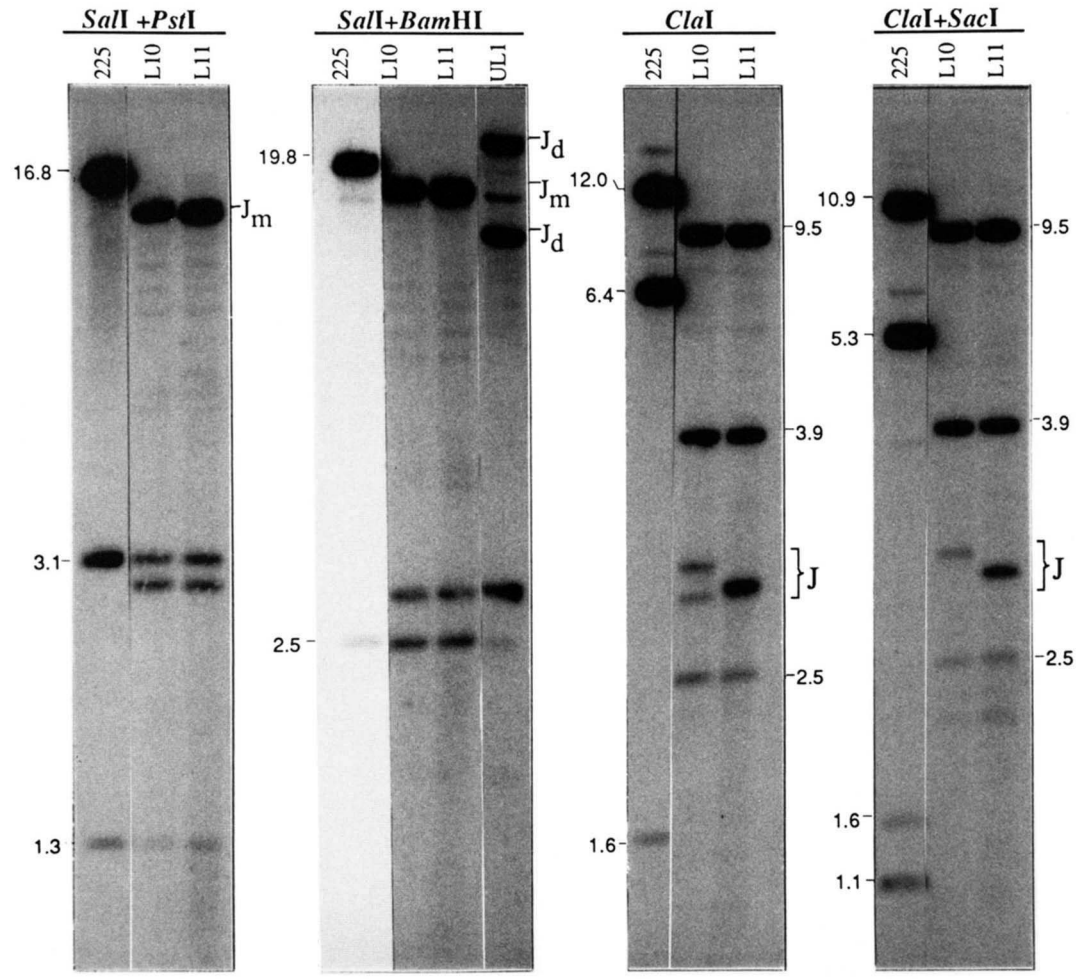

FIGURE 5.—DNA gel-transfer hybridization analysis of yeast transformants resulting with a plasmid cleaved within a *lacZYA* inverted repeat. DNA isolated from two Leu$^+$ yeast transformants, L10 and L11, and one Ura$^+$ Leu$^+$ transformants, UL1, resulting after transformation with a *Sac*I-cleaved mixture of pSK225 and pSK227 (Figure 4), was visualized after the indicated restriction digestion. Plasmid pSK225 was included as a control. Hybridization was performed with radioactively labeled pSK292, a derivative of pSK225 lacking the *URA3* insert. End-to-end junction restriction fragments specifically generated by the expected monomeric product of transformation (Figure 4) are denoted J_m. Junction restriction fragments expected on the presence of the head-to-head dimer product (Figure 4) are denoted J_d. The sizes of other plasmid restriction fragments are indicated in kilobases. The restriction maps of pSK225, the dimeric product and the monomeric product, are shown in Figure 4.

the shorter molecule. In the remaining plasmid, a junction containing a single *Eco*RI site was detected. The analysis of the junction structure of some of these plasmids was extended after their transfer to *E. coli*. On account of the possible instability of inverted dimer plasmids propogated in *E. coli* (see KUNES, BOTSTEIN and FOX 1985), the junction fragments of plasmid DNA isolated from *E. coli* was compared directly by gel-transfer hybridization analysis with the plasmid of the corresponding yeast transformant. No differences were detected. The restriction analysis of the three plasmids shown in Figure 6 is consistent with this conclusion. The proposed junction structure of these plasmids was also confirmed by DNA sequence analysis (manuscript in preparation). Finally, the presence of both the *URA3* and *LEU2* markers was confirmed directly for each of the 21 plasmids (see, for example, the restriction analysis in Figure 6). Thus, for the most part, fusion of the shortened pSK136 molecule with the longer pSK142 molecule is accompanied by either loss of the extra pSK142 material, or replacement of the missing pSK136 material. The resulting junction is thus highly symmetrical.

In principle, a recombinant product would be produced if a shorter molecule acquires extra material by recombination with a longer end. To determine whether this is the case, a plasmid was constructed such that, during an intramolecular fusion event, recombination with a longer plasmid end would generate from two heteroallelic *ura3* genes a *URA3* recombinant. Plasmid pSK218 (Figure 8) is an autonomous yeast plasmid containing the *LEU2* gene and two mutant copies of the yeast *URA3* gene in inverted orientation, separated by a 1-kb DNA fragment (referred to as the spacer). One *ura3* gene is marked by *ura3-3*, a nonsense mutation located at the amino

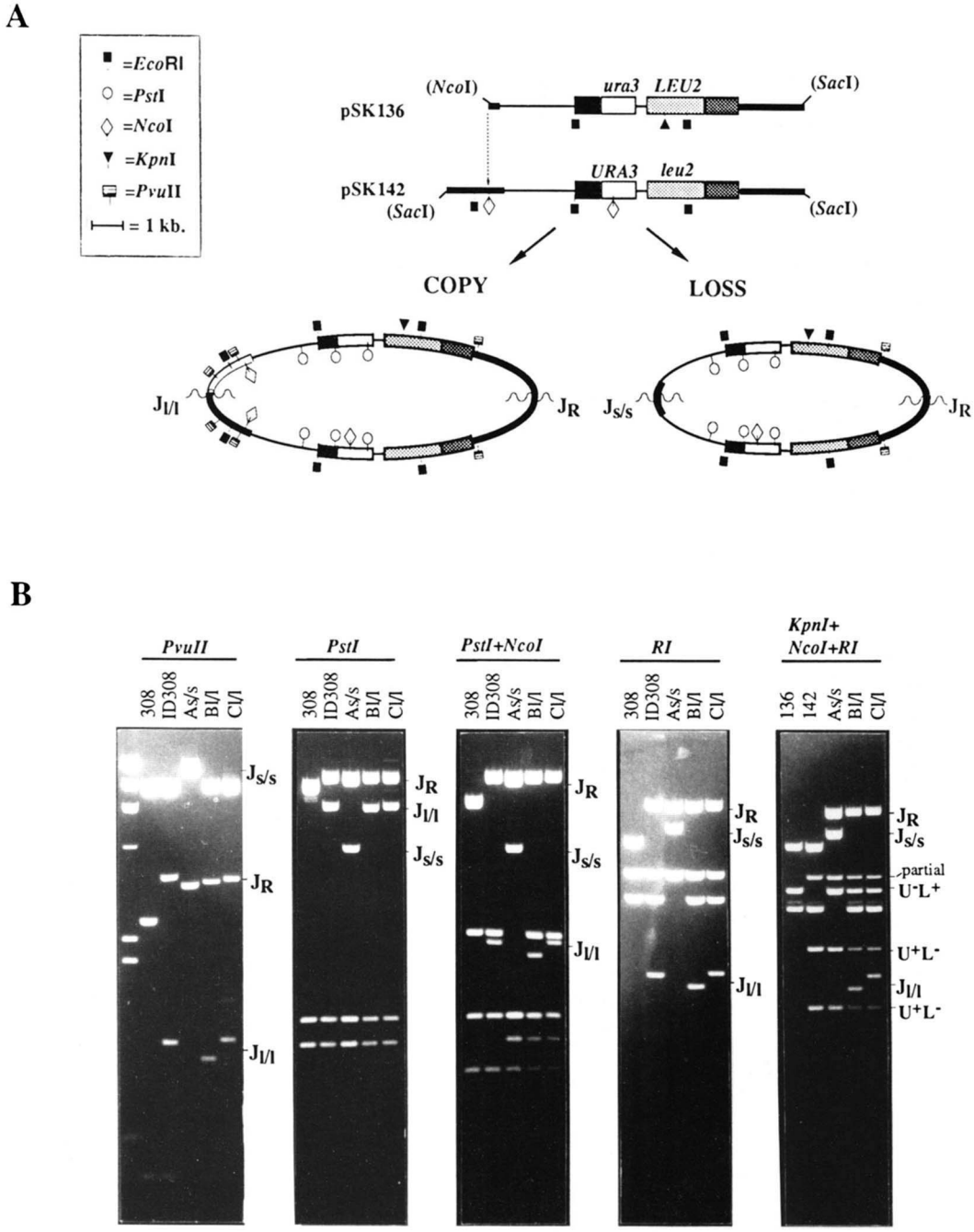

FIGURE 6.—Inverted dimer plasmids formed by joining plasmids of unequal length. A, Plasmid pSK142 (*URA3 leu2*) is shown in *Sac*I-linearized form. This molecule is 1.3 kb longer than the *Nco*I, *Sac*I generated molecule of pSK136 (*ura3 LEU2*) also shown. An equimolar mixture of these two DNA molecules, purified after electrophoresis in an agarose gel, was used to transform yeast strain DBY1226 (*ura3 leu2*), with selection for Ura^+ or Leu^+ transformants. Transformants with the Ura^+ Leu^+ phenotype were then analyzed by DNA gel-transfer hybridization (see Figure 7). With rare exception, these transformants harbor a plasmid belonging to one of the two classes shown below the arrows. On the left, the Ura^+ Leu^+ plasmid product has a junction in which the 1.3-kb segment excised from pSK136 has been replaced, presumably by copy of a longer pSK142 molecule end prior to fusion. The resulting junction is designated $J_{l/l}$. On the right is illustrated the second class of Ura^+ Leu^+ plasmid products, in which the extra material of the longer pSK142 molecule has been lost prior to fusion with pSK136. The resulting junction is designated $J_{s/s}$. B, A restriction analysis of three Ura^+ Leu^+ plasmids isolated after yeast transformation with the mixture of two plasmid DNAs described in A. Plasmid DNA was prepared after isolation of the plasmids in *E. coli*, and cleaved with the indicated restriction enzymes. Included as controls are *Hin*dIII-cleaved phage λ DNA, pCH308 and ID308, an inverted dimer plasmid formed with *Sac*I-cleaved pCH308 (as shown in Figure 1), pSK136, and pSK142 cleaved with the indicated restriction enzyme. Two of the plasmids, *Bl/l* and *Cl/l*, bear the $J_{l/l}$ junction described in A. The remaining plasmid, *As/s*, bears the $J_{s/s}$ junction described in A. The bands corresponding to the junction-containing fragments are indicated. The fragments corresponding to the rightward junction, J_R (see A), are also indicated. The plasmid's *URA3* and *LEU2* genotypes are revealed by the *Nco*I and *Kpn*I cleavages, respectively (see Figure 3 for details of the analysis of plasmid genotype). The informative restriction fragments are indicated as follows: U^+L^-, *URA3 leu2*; U^-L^+, *ura3 LEU2*; U^+, *URA3*; U^-, *ura3*.

TABLE 3

Formation of heterozygous inverted dimer plasmids from linearized plasmids of different length

Primary selection	Fraction Ura$^+$ Leu$^+$/total with various amounts of DNA (ng)			
	40	80	160	320
Ura$^+$	29/98	31/48	24/49	36/52
Leu$^+$	38/103	26/51	22/51	29/48

Yeast strain DBY1226 (*ura3 leu2*) was transformed with an equimolar mixture of *Sac*I-cleaved pSK142 (*URA3 leu2*) and *Nco*I, *Sac*I-cleaved pSK136 (*ura3 LEU2*). Both linearized plasmid DNAs were recovered by electroelution after electrophoresis in an agarose gel. The table gives the fraction of transformants obtained on either Ura$^+$ or Leu$^+$ primary selection which display the Ura$^+$ Leu$^+$ phenotype (Ura$^+$ Leu$^+$/total).

terminal end of the gene. The other is deleted for the carboxyl terminal 200 bp of coding sequence, where the deletion end is joined to the spacer fragment at a unique *Sma*I restriction site. As shown in Figure 8, the *ura3* deletion end of the *Sma*I-linearized plasmid is shorter than the homologous end containing the inverted *ura3-3* gene and the 1-kb spacer. Recombination that restores the *ura3* deletion end with the wild-type information from the longer *ura3-3* end would generate a wild-type *URA3* gene and yield a Ura$^+$ transformant. On the other hand, loss of the extra material of the longer end of *Sma*I-linearized pSK218 would produce a junction in which both copies of *ura3* were deleted at their carboxyl ends. In yeast harboring pSK218, recombination between the two *ura3* genes generates *URA3* recombinants at a frequency of about 1%, resulting in a Ura$^+$ papillation phenotype on media lacking uracil. A product in which both *ura3* genes bear 3′ deletions should fail to papillate Ura$^+$.

With *Sma*I-linearized pSK218, Leu$^+$ transformants of DBY1226 (*leu2 ura3-3*) were obtained with a first order DNA concentration dependence (data not shown), consistent with repair by an intramolecular fusion reaction. At all of the DNA concentrations, about 30% of the Leu$^+$ transformants were found to be Ura$^+$ (Table 4). Approximately 60% of the Leu$^+$ transformants failed to display the original pSK218 Ura$^+$ papillation phenotype; they were stably Ura$^-$. The remaining 10% of the transformants papillated Ura$^+$ at about the frequency of pSK218. On the other hand, transformation with closed circular pSK218 resulted in Leu$^+$ transformants that rarely (1%) showed the Ura$^+$ phenotype. The Ura$^-$ phenotype was never detected.

Each of eight Ura$^+$ Leu$^+$ transformants examined by gel-transfer hybridization contained a monomer plasmid product, as expected (Figure 7). In each case, the restriction cleavage pattern was consistent with the addition to the *ura3* deletion end of the missing *URA3* material and most of the 1 kb spacer fragment. Hence the recombinant *URA3* product has a symmetrical fusion junction in the spacer fragment region. All but one of 18 nonpapillating Ura$^-$ Leu$^+$ transformants had a junction formed apparently after loss of the extra length of material from the *ura3-3* plasmid end. The remaining case had a junction similar in

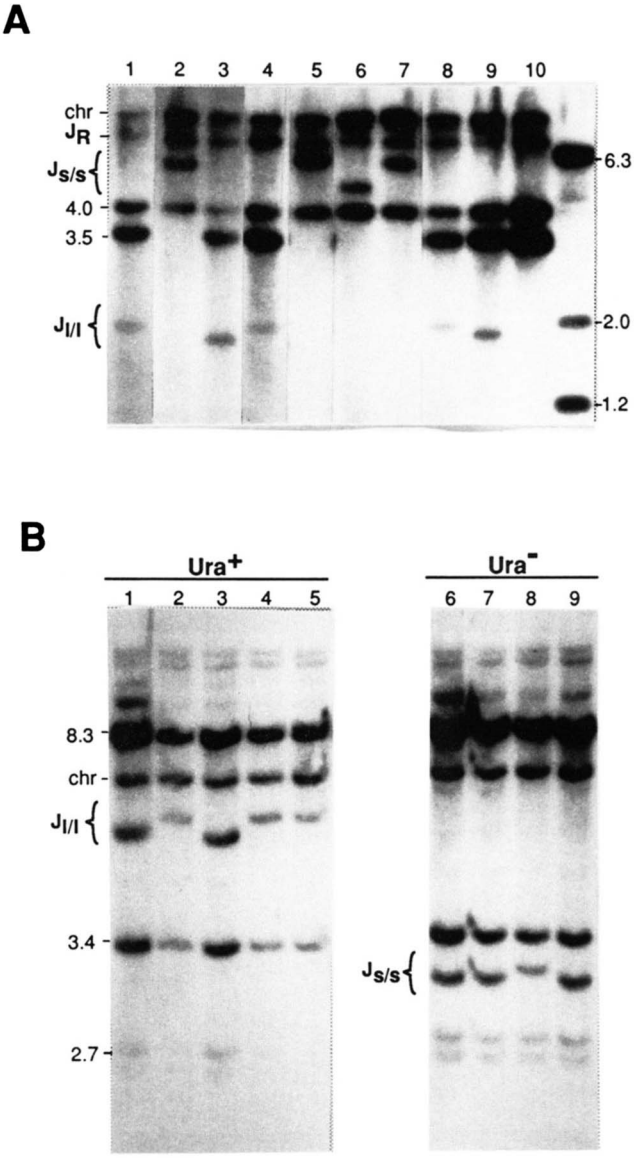

FIGURE 7.—DNA gel-transfer hybridization analysis of transformants recovered after transformation with linearized plasmids of unequal length. A, DNA isolated from 10 Ura$^+$ Leu$^+$ transformants resulting with the mixture of the short *Nco*I, *Sac*I-generated pSK136 molecule and the long *Sac*I-generated pSK142 molecule (Figure 6A), was cleaved with *Eco*RI and visualized by gel-transfer hybridization. The plasmid *Eco*RI restriction fragments, of 3.5 kb, 4.0 kb, and the junction fragments, $J_{s/s}$, $J_{l/l}$, and J_R, are indicated. See Figure 6A for the *Eco*RI restriction map of the dimer products bearing $J_{s/s}$ and $J_{l/l}$. Also, shown for comparison in Figure 6B are the *Eco*RI cleavage products of three dimer plasmids bearing either $J_{s/s}$ or $J_{l/l}$. B, DNA from 5 Ura$^+$ Leu$^+$ and 4 Ura$^-$ Leu$^+$ transformants resulting with *Sma*I-linearized pSK218 (Figure 8) was cleaved with *Pvu*I and visualized by gel-transfer hybridization. Indicated are the positions of bands consistent with the presence of the Ura$^+$ $J_{l/l}$ and Ura$^-$ $J_{s/s}$ junctions. For comparison, see the *Pvu*I cleavage products of the Ura$^+$ and Ura$^-$ products isolated in *E. coli*. The bands corresponding the remaining pSK218 *Pvu*I fragments are indicated by their size (in kb).

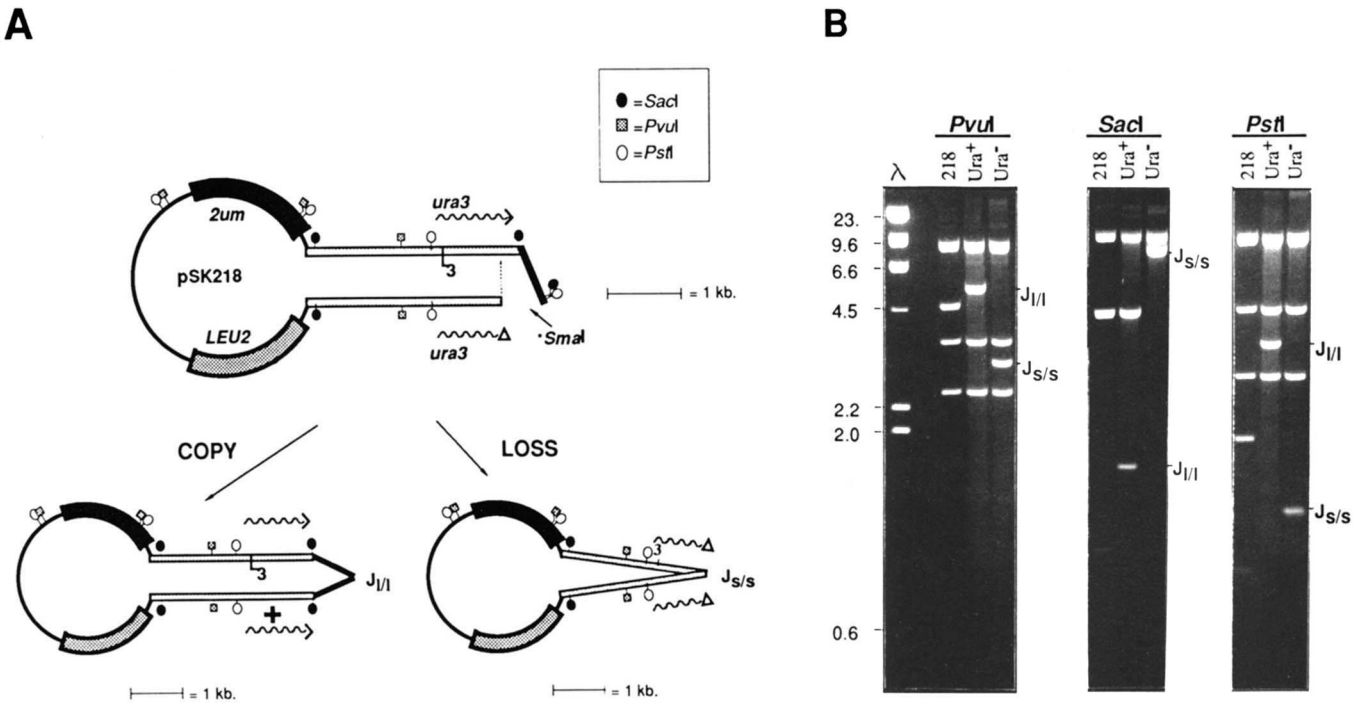

FIGURE 8.—Generation of *URA3* recombinant plasmids by fusion at heteroallelic *ura3* ends of unequal length. Plasmid pSK218 harbors two inverted, heteroallelic, *ura3* genes separated by a 1-kb asymmetric DNA (spacer) fragment. One *ura3* copy is marked at the amino end of the coding sequence by the *ura3-3* (am) mutation (nucleotide 245; ROSE, GRISAFI and BOTSTEIN 1983). The second *ura3* copy is deleted at the coding sequence's carboxyl end (nucleotide 882; ROSE, GRISAFI and BOTSTEIN 1983), where a *Sma*I-linker joins it to the asymmetric spacer. As described in the text, after transformation with *Sma*I-linearized pSK218, copy of the longer *ura3-3* end prior to end-fusion will generate a *URA3* recombinant plasmid. Loss of the longer end will generate a plasmid bearing carboxy terminal deletions in both *ura3* genes. B, Restriction analysis of Ura$^+$ and Ura$^-$ plasmid products resulting after yeast transformation with *Sma*I-linearized pSK218. Two plasmids, one from a Ura$^+$ yeast transformant and one from a Ura$^-$ yeast transformant, were recovered in *E. coli*. Plasmid DNA isolated from *E. coli* was cleaved with the indicated restriction enzyme. Included as controls are pSK218 DNA and phage λ DNA cleaved with *Hin*dIII. The positions of the bands corresponding to the junction fragments $J_{I/I}$ and $J_{S/S}$ are indicated.

TABLE 4

Formation of *URA3* recombinants by intramolecular fusion of a plasmid with inverted unequal *ura3* ends

	Phenotype of Leu$^+$ transformants resulting with various amounts of *Sma*I-linearized pSK218 DNA (ng)			
Phenotype	10	20	80	320
Ura$^+$ Leu$^+$	30	27	13	38
Ura$^+$ (pap.) Leu$^+$	7	9	5	6
Ura$^-$ Leu$^+$	67	76	42	60
Total Leu$^+$ tested	104	112	60	104

Yeast strain DBY1226 was transformed with various amounts of *Sma*I-linearized pSK218 (Figure 8). The table gives the number of Leu$^+$ transformants recovered in the presence of uracil that displayed the Ura$^+$, Ura$^-$ or Ura$^+$ papillation (pap.) phenotype.

structure to the Ura$^+$ transformants examined above. We presume that this plasmid had become homozygous *ura3-3*. Restriction analysis of several *URA3* and *ura3* plasmids after rescue in *E. coli* (Figure 8) supports these conclusions.

DISCUSSION

In order to distinguish amongst several possible mechanisms for inverted dimer plasmid formation, we determined the number of linearized plasmid molecules required in the formation of each plasmid. Most, if not all, inverted dimer plasmids result from the joining of two linearized plasmid molecules. This conclusion is based on the observation of a bimolecular DNA concentration requirement for the repair of linearized plasmid DNA, except on transformation at very low plasmid concentrations, and on the outcome of transformation with genetically marked plasmids. Heterozygous and homozygous inverted dimer plasmids occur with the same DNA concentration dependence. Among inverted dimer plasmids isolated by selection for a single marker, heterozygous dimer plasmids are present at a frequency close to that expected. With a mixture of three marked plasmids, the products rarely display all three markers. This outcome shows that the recovery of heterozygotes is not due to promiscuous reassortment of the markers in a large freely recombining pool of molecules.

At low DNA concentrations, however, a substantial portion of the transformants harbor the monomeric products of an apparently inefficient head-to-tail joining reaction. This could be the same mechanism that forms the monomeric products recovered in the absence of carrier DNA (ORR-WEAVER and SZOSTAK

1983; KUNES, BOTSTEIN and FOX 1985). Indeed, the increased yield of transformants obtained by including highly fragmented carrier DNA is consistent with the resulting fraction of transformants found to harbor inverted dimer plasmids. We estimate that, at DNA saturation, bimolecular head-to-head joining is 20–100-fold more likely than the apparent unimolecular reaction. On the basis of the relative yield of transformants with closed circular plasmid DNA, and a plasmid that undergoes intramolecular repair utilizing inverted homologous ends, we estimate a probability of at least 10% for the joining of a single pair of homologous ends.

Taken together, these results argue strongly against the formation of the dimer plasmids by a simple unimolecular mechanism; that of hairpin-loop formation at both duplex ends of a single plasmid molecule, and subsequent replication. Why then might two plasmid molecules join more efficiently, preferring to join at homologous ends? The possibility that this is due to the action of a site-specific recombinase at particular DNA sequences is not consistent with the prominence of head-to-head joinings from plasmids cleaved in different regions of DNA (KUNES, BOTSTEIN and FOX 1985) or with the formation of end-to-end junctions at any of a large number of nucleotide sites (our unpublished observations). We therefore investigated a possible role for homologous pairing in aligning the molecules. The requirement of two plasmid molecules for repair would thus reflect the rate-limiting role of homologous pairing in the end-joining reaction.

This notion is supported by the following observations. First, a plasmid with homologous but inverted ends, which can thus undergo head-to-tail homologous pairing, is repaired efficiently in an intramolecular reaction. This plasmid transforms yeast with a unimolecular DNA concentration dependence. In nearly every transformant analyzed, the resident plasmid had the structure expected of a head-to-tail joining product. Second, we have provided evidence which suggests that plasmid end-to-end joining can be accompanied by homologous recombination. Plasmids that result from joining a *TRP1* linearized plasmid with a *trp1* linearized plasmid were *trp1/trp1* homozygotes 8% of the time. *TRP1/TRP1* homozygotes might be expected with equal frequency. Less than 1% of the transformants resulting with a closed circular *TRP1/trp1* inverted dimer plasmid were Trp^-, which implies that recombination is dependent on the presence of free DNA ends. In preliminary experiments with other plasmid markers, we find that increasing a marker's proximity to the plasmid's free ends increases the frequency of recombinants. This is also consistent with a primary role of free DNA ends in initiating the reaction. Third, in the joining of two linearized plasmids of different length, the material absent from a shortened plasmid end can be restored with information gained by recombination with the longer plasmid. This is clearly demonstrated by the formation of a *URA3* recombinant product from the fusion of heteroallelic *ura3* ends, where a terminal *ura3* deletion end acquires wild-type information from a longer *ura3* end. This reaction bears some resemblance to the gap-repair reaction that takes place on integration of a linearized plasmid at a homologous chromosomal locus (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981). However, the alternative outcome, that of shortening the longer plasmid end prior to joining with the shorter plasmid, is not readily explained in these terms.

The precise nature of the joining reaction remains unclear. The role of homologous pairing could be to sequester a pair of free DNA ends into the close proximity that may be necessary for joining. Nonhomologous ends would thus be less likely to join if they were to associate only with the lower probability of random collision. This hypothesis suggests that head-to-tail joining efficiency might increase with smaller plasmids. A second possibility is that homologous pairing might act to locally increase the concentration of a participant in the end-joining reaction, for example, an enzyme shared in the machinery of homologous and nonhomologous recombination. A third possibility is that homologous pairing between two free plasmid ends generates a reactive substrate for a second reaction that fuses the ends. One such possible mechanism, illustrated in Figure 9, relies on the notion that a replication fork, initiated by homologous strand transfer, stalls as it approaches a free duplex end. The template duplex may rewind and displace the newly-synthesized strand, which could form a novel DNA joint by participating in aberrant basepairing followed by DNA synthesis or ligation events.

To what extent, if any, does the inverted dimer repair pathway mirror the processing of chromosomal DNA double-chain breaks? Synapsis-mediated fusion of chromosomal DNA ends could occur after breakage of homologous chromosomes at similar positions, or after replication of a single chromosomal fragment produces homologous sister fragments. Joining of these sister ends would form symmetrical chromosomal products (Figure 10). An attempt has been made to detect such products of chromosomal breakage in yeast (HABER and THORBURN 1984; HABER, THORBURN and ROGERS 1984). Chromosomal breakage occurred during the mitotic segregation of a dicentric chromosome formed by meiotic recombination. Most of the resulting rearrangement products could be accounted for by homologous recombination between broken ends and a duplicated region of the dicentric chromosome. A minority of the products acquired new stable chromosomal ends at novel posi-

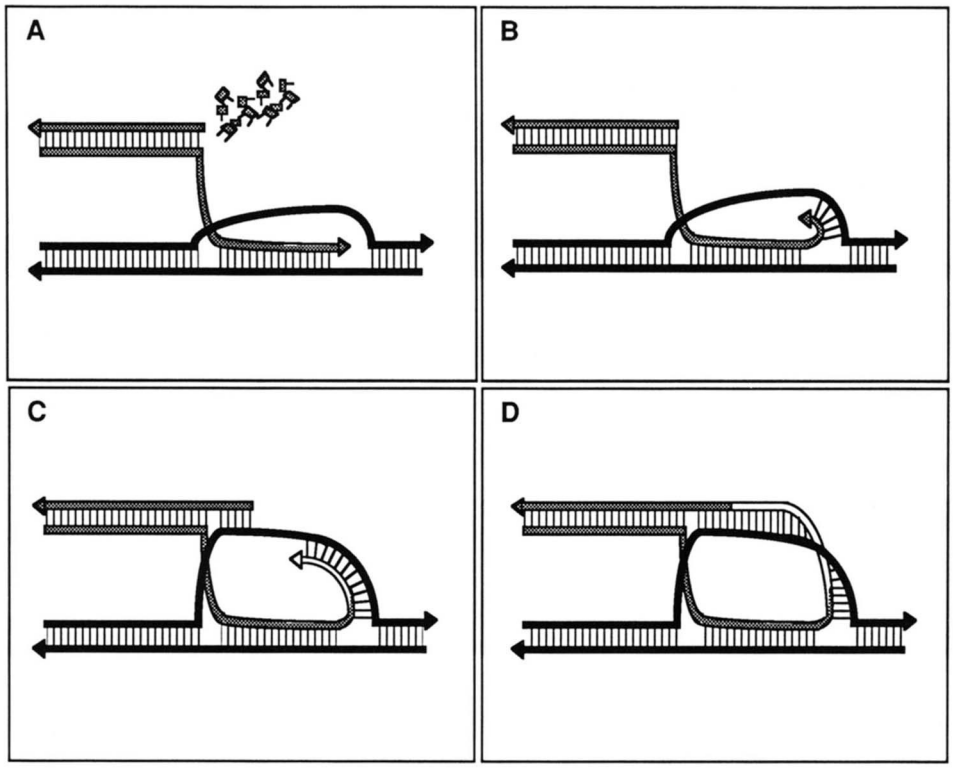

FIGURE 9.—Possible mechanism of synapsis-mediated end-fusion. The cartoon depicts a possible mechanism in which an invading single DNA strand serves as a primer for DNA synthesis. The replication fork stalls as it nears the end of the template, and the priming strand is displaced by template duplex rewinding. Mispairing of the displaced strand, continued DNA synthesis, and ligation to the second invading end, generate the closed loop structure shown in D. The Holliday structure, when resolved by cleavage in either possible plane, would yield a joint molecule that, on replication, bears a head-to-head dimer junction.

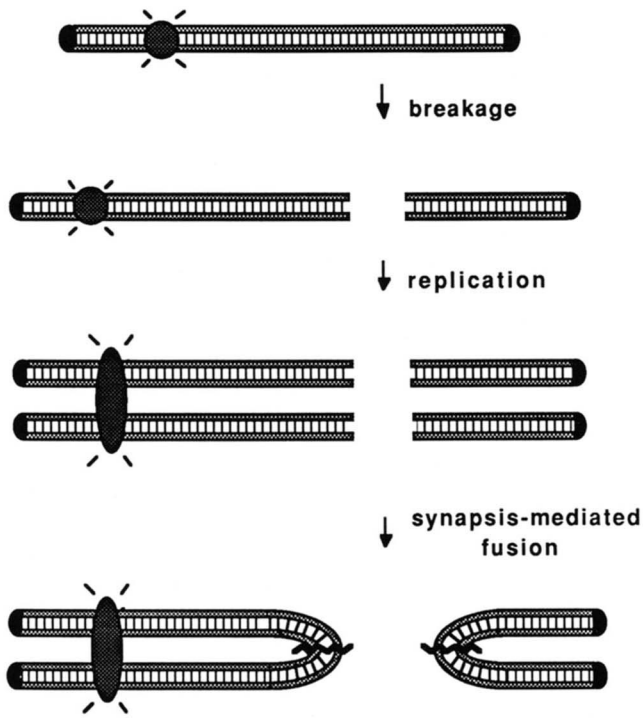

FIGURE 10.—A possible role for synapsis-mediated fusion in rearrangement mechanisms: The bridge-breakage fusion cycle of *Zea mays* was described by MCCLINTOCK (1939). The products of chromosomal breakage, a centromere-containing fragment and an acentric fragment, are processed to yield, after DNA replication, linear head-to-head dimers of the original chromosomal fragments. A novel end-to-end junction is generated at the sites of the fragment's original free chromosomal ends. As illustrated, this outcome could be accounted for by synapsis-mediated fusion of the sister chromatid fragments.

tions, like the "healing" events detected in maize by MCCLINTOCK (1941). In contrast to the observations in yeast, symmetrical chromosomal products are formed after chromosome breakage in maize (MCCLINTOCK 1939). Here, the dicentric symmetrical product breaks again at the next mitotic anaphase, with the result that the process of breakage, fusion and breakage is cyclical. Rearrangements that result in gene amplification also might form in a reaction related to inverted dimer plasmid formation. Analysis of the structure of the repeated gene arrays at an amplified locus implicates the formation of an inverted gene duplication as a first step in the amplification process (FORD and FRIED 1986).

A quiescent DNA end-processing mechanism that can be induced by highly fragmented carrier DNA during transformation might be otherwise induced to act on chromosomal double-chain breaks by extensive genomic DNA damage. The large number of free DNA ends provided by carrier DNA might mimic the presence of a large number of chromosomal double-chain breaks. Such a signal would indicate for the cell that it is unlikely that an intact homolog, a substrate for recombinational repair, has survived. Pairing of broken sister chromatids might occur in a manner that would ordinarily result in recombinational repair were one chromatid intact. With both chromatids broken, symmetrical end-to-end junction formation might prove to be the only alternative to have otherwise dead-end pathway. These considerations suggest that an appropriately devised attempt to detect such

chromosomal rearrangements in yeast may yet prove fruitful.

This work was supported by a grant from the National Institutes of Health to M.S.F. (AI05388). We thank M. SANDER (M.I.T.) for critical reading of the manuscript.

LITERATURE CITED

ANDREADIS, A., Y.-P. HUS, G. B. KOHLHAW and P. SCHIMMEL, 1982 Nucleotide sequence of yeast *LEU2* gene shows 5′-noncoding region has sequences cognate to leucine. Cell **31:** 319–325.

CLEWELL, D. B., and D. R. HELINSKI, 1969 Supercoiled circular DNA protein complex in *E. coli*: purification and induced conversion to an open circular DNA form. Proc. Natl. Acad. Sci. USA **62:** 1159–1166.

FALCO, S. C., Y. LI, J. R. BROACH and D. BOTSTEIN, 1982 Genetic consequences of chromosomally integrated 2 μm plasmid in yeast. Cell **29:** 573–584.

FORD, M., and M. FRIED, 1986 Large inverted duplications are associated with gene amplification. Cell **45:** 425–430.

HABER, J. E., and P. C. THORBURN, 1984 Healing of broken linear dicentric chromosomes in yeast. Genetics **106:** 207–226.

HABER, J. E., P. C. THORBURN and D. ROGERS, 1984 Meiotic and mitotic behavior of dicentric chromosomes in *Saccharomyces cerevisiae*. Genetics **106:** 185–206.

HINNEN, A., J. B. HICKS and G. R. FINK, 1978 Transformation of yeast. Proc. Natl. Acad. Sci. USA **75:** 1929–1933.

HOLM, C., D. W. MEEKS-WAGNER, W. L. FANGMAN and D. BOTSTEIN, 1986 A rapid, efficient method for isolating DNA from yeast. Gene **42:** 169–173.

KUNES, S., D. BOTSTEIN and M. S. FOX, 1985 Transformation of yeast with linearized plasmid DNA: formation of inverted dimers and recombinant plasmid products. J. Mol. Biol. **184:** 375–387.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

MCCLINTOCK, B., 1939 The behavior in successive nuclear divisions of a chromosome broken at meiosis. Proc. Natl. Acad. Sci. USA **25:** 405–416.

MCCLINTOCK, B., 1941 The stability of broken ends of chromosomes in *Zea mays*. Genetics **26:** 234–282.

MILLER, J. H., 1972 *Experiments in Molecular Genetics.* Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

ORR-WEAVER, T. L., and J. W. SZOSTAK, 1983 Yeast recombination: the association of between double-strand gap repair and crossing-over. Proc. Natl. Acad. Sci. USA **80:** 4417–4421.

ORR-WEAVER, T. L., J. W. SZOSTAK and R. J. ROTHSTEIN, 1981 Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78:** 6354–6358.

RADLOFF, R., W. BAUER and J. VINOGRAD, 1967 A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in HeLa cells. Proc. Natl. Acad. Sci. USA **57:** 1514–1521.

RAMBACH, A., and D. S. HOGNESS, 1977 Translation of *Drosophila melanogaster* sequences in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **74:** 5041–5045.

ROSE, M., and D. BOTSTEIN, 1983 Structure and function of the yeast *URA3* gene. Differentially regulated expression of hybrid beta-galactosidase from overlapping coding sequences in yeast. J. Mol. Biol. **170:** 883–904.

ROSE, M., P. GRISAFI and D. BOTSTEIN, 1983 Structure and function of the yeast *URA3* gene: expression in *Escherichia coli*. Gene **29:** 113–124.

ROTHSTEIN, R. J., 1983 One-step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

SHERMAN, F., G. R. FINK and C. W. LAWRENCE, 1979 *Methods in Yeast Genetics.* Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

SOUTHERN, E. M., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.

STRUHL, K., 1985 Nucleotide sequence and transcriptional mapping of the yeast *PET56-HIS3-DED1* gene region. Nucleic Acids Res. **13:** 8587–8601.

SUZUKI, K., Y. IMAI, I. YAMASHITA and S. FUKUI, 1983 *In vivo* ligation of linear DNA molecules to circular forms in the yeast *Saccharomyces cerevisiae*. J. Bacteriol. **155:** 747–754.

THALER, D. S., and F. W. STAHL, 1988 DNA double-chain breaks in recombination of phage λ and yeast. Annu. Rev. Gen. **22:** 169–198.

TSCHUMPER, G., and J. CARBON, 1979 Sequences of a yeast DNA fragment containing a chromosomal replicator and the *TRP1* gene. Gene **10:** 157–166.

Communicating editor: J. R. ROTH