Formation of Large Palindromic DNA by Homologous Recombination of Short Inverted Repeat Sequences in *Saccharomyces cerevisiae*

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ABSTRACT

Large DNA palindromes form sporadically in many eukaryotic and prokaryotic genomes and are often associated with amplified genes. The presence of a short inverted repeat sequence near a DNA double-strand break has been implicated in the formation of large palindromes in a variety of organisms. Previously we have established that in *Saccharomyces cerevisae* a linear DNA palindrome is efficiently formed from a single-copy circular plasmid when a DNA double-strand break is introduced next to a short inverted repeat sequence. In this study we address whether the linear palindromes form by an *inter*molecular reaction (that is, a reaction between two identical fragments in a head-to-head arrangement) or by an unusual *intra*molecular reaction, as it apparently does in other examples of palindrome formation. Our evidence supports a model in which palindromes are primarily formed by an intermolecular reaction involving homologous recombination of short inverted repeat sequences. We have also extended our investigation into the requirement for DNA double-strand break repair genes in palindrome formation. We have found that a deletion of the *RAD52* gene significantly reduces palindrome formation by intermolecular recombination and that deletions of two other genes in the *RAD52*-epistasis group (*RAD51* and *MRE11*) have little or no effect on palindrome formation. In addition, palindrome formation is dramatically reduced by a deletion of the nucleotide excision repair gene *RAD1*.

LARGE DNA palindromes have been observed in the genomes of prokaryotes and in the nuclear and organellar genomes of protozoa, fungi, plants, and animals (see Fried *et al.* 1991 and Rayko 1997 for reviews). In some organisms, large palindromes form as a result of a precisely regulated developmental program. In *Tetrahymena thermophila*, for example, a large palindrome encompassing the ribosomal RNA gene forms in all cells undergoing development of the macronucleus (Yao *et al.* 1985). For most organisms, however, large palindromes are not formed as a normal part of the life cycle, but rather arise sporadically as an abnormal event in a rare subpopulation of cells. In yeast, parasitic protozoa, and cultured mammalian cells, palindromes have been found only in cells selected for resistance to a variety of cytotoxic agents (Ford *et al.* 1985; Ford and Fried 1986; Nalbantoglu and Mueth 1986; Walton *et al.* 1986; Hyrien *et al.* 1988; Ouellette *et al.* 1991; Huang and Campbell 1995; Albrecht *et al.* 2000). Of particular importance to human health, large palindromic structures, typically including an oncogene, have been observed late in tumor progression (Ford *et al.* 1985; Ford and Fried 1986). Most palindromic structures become highly amplified and it is thought that the formation of a large palindrome is a critical, early step in some pathways of gene amplification (Fried *et al.* 1991).

The unexpectedly common association of palindromic DNA with gene amplification has stimulated interest in understanding how large DNA palindromes form. Several studies have implicated the occurrence of a DNA double-strand break near short inverted repeat sequences as the starting point in the formation of large DNA palindromes (Yasuda and Yao 1991; Bi and Lui 1996; Butler *et al.* 1996; Albrecht *et al.* 2000; Qin and Cohen 2000; Lobachev *et al.* 2002). One possible mechanism by which a palindrome can form from such a precursor involves a short inverted repeat sequence guiding the formation of a hairpin at the broken end; the other end is capped by a telomere. Bidirectional DNA replication converts the giant hairpin molecule to a palindrome (see Figure 1A for an example of how a linear palindrome can form). This mechanism can be regarded as *intra*molecular since the palindrome is derived from a single precursor molecule. There is strong support for an intramolecular mechanism underlying the developmentally regulated formation of rDNA palindromes in Tetrahymena (Yasuda and Yao 1991; Butler *et al.* 1995). It is not clear whether such an intramolecular mechanism occurs widely among different organisms or is more limited in scope. A second possible mechanism, also arising from the presence of short inverted repeats at a broken end, is *inter*molecular in nature. If two copies of the precursor molecule are in the same cell, the ter-

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minal short inverted repeat sequences can mediate a homologous recombination reaction that brings together the two identical molecules in a head-to-head arrangement, thus forming a palindrome (see Figure 1B). In principle, short inverted repeats of any sequence can function in either mechanism of palindrome formation, since it is the symmetrical structure of an inverted repeat and not its sequence *per se* that is critical for mediating intra- or interstrand pairing.

In organisms where large palindromes occur sporadically it is generally difficult to study their formation due to the rarity of the relevant events. To circumvent this problem we have developed a system for investigating the formation of large DNA palindromes in the budding yeast *Saccharomyces cerevisiae* (Butler *et al.* 1996). In this system, a palindrome is efficiently generated from a single-copy plasmid when a double-strand break, catalyzed by the site-specific HO endonuclease, is introduced near a 42-bp inverted repeat sequence. This reaction is strictly dependent on this short inverted repeat sequence and the nearby double-strand break. As a result of this reaction, the short inverted repeat sequence becomes the exact center of symmetry in the palindromic molecule.

In this study we have investigated the molecular mechanism of palindrome formation in Saccharomyces. We have designed two different assays to distinguish between the intra- and intermolecular models of palindrome formation. Our evidence supports a model in which palindromes are primarily formed by an intermolecular reaction involving homologous recombination of short inverted repeat sequences. We have also extended our analysis of the role of DNA double-strand break repair genes in palindrome formation. We have found that a deletion of the *RAD1* gene or the *RAD52* gene dramatically reduces palindrome formation by intermolecular recombination, whereas a deletion of either the *RAD51* gene or the *MRE11* gene has little or no effect on palindrome formation.

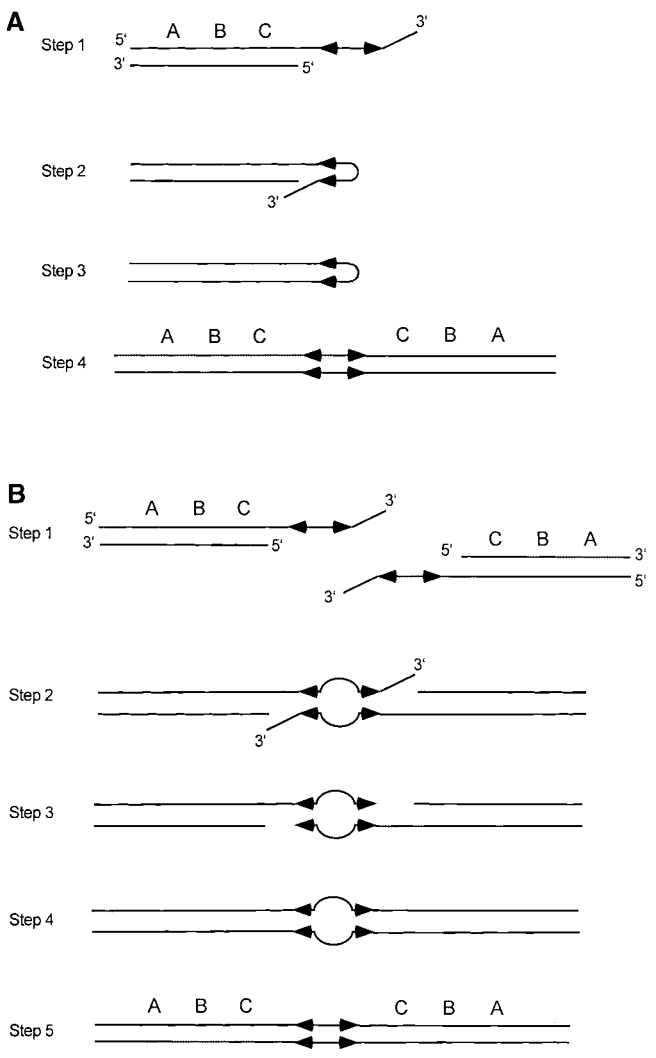


Figure 1.—Possible intra- and intermolecular models of palindrome formation. Both strands of relevant DNA molecules are shown. The short inverted repeat sequence is represented by the double arrowheads. The uppercase letters represent the orientation of hypothetical genetic markers on the precursor molecules and the palindromic product. Both models begin with double-strand breaks having occurred near the short inverted repeat sequence. (A) Intramolecular recombination model. Step 1, 5′ to 3′ resectioning of the DSB to yield a 3′ single-stranded overhang that includes the short inverted repeat sequence. Step 2, intrastrand annealing within the short inverted repeat sequence. Step 3, removal of 3′ nonhomologous tail, DNA synthesis to fill in any gap and ligation result in a giant hairpin molecule. Step 4, bidirectional DNA replication originating within the giant hairpin forms the mature palindrome. (B) Intermolecular recombination model. Step 1, 5′ to 3′ resectioning of the DSBs to yield 3′ single-stranded overhangs that include the short inverted repeat sequence. Step 2, annealing of single strands at the short inverted repeat sequence brings the two molecules in a head-to-head arrangement. Note that the initial annealing reaction can occur between the repeats only; the sequence between the arrows is nonpalindromic and therefore noncomplementary. Thus, the initial annealed intermediate will have a 28-bp "bubble." Step 3, removal of 3′ nonhomologous tails leaves two gaps. Step 4, DNA synthesis to fill in the gaps and ligation to seal the nicks. Step 5, conventional replication of this molecule will form two fully base-paired palindromes (only one is shown). Both of these models are based on a form of homologous recombination known as single-strand annealing (Lin *et al.* 1984). Models of palindrome formation based on other recombination mechanisms are also possible (see discussion).

MATERIALS AND METHODS

Strains and plasmids: The construction of p42IRURA has been described (Butler *et al.* 1996). p42IRLEU was constructed by inserting a 4.1-kbp blunt-end fragment containing the *LEU2* gene into a unique *Nco*I site located in the *URA3* gene of p42IRURA. To construct pDIR001 we isolated a 300-bp *Bam*HI fragment derived from p42IRURA that contains a complete copy of the short inverted repeat sequence (42-bp repeats, separated by a 28-bp spacer). The single-strand overhangs were removed by T4 DNA polymerase and the resulting blunt-ended

fragment was inserted into a site 20 bp to the right of the "right" HO endonuclease cut site (cs) of p42IRURA. The site adjacent to the right HO cs was created by digesting p42IRURA with *Eco*RI and *Xba*I (which deletes the $C_{1-3}A$ tract) and removing the single-stranded overhangs with T4 DNA polymerase. All strains used in this study are derived from LS20 (Sandell and Zakian 1993). The genotype of LS20 is *mat*Δ, *his3*, *ade2*, *can1*, *trp1*, *ura3*, *leu2*, *lys5*, *cyh*^{r}, *ade3::GalHO*. The Rad$^+$ ATB996 strain was made by transformation of LS20 with p42IRURA and p42IRLEU. All gene disruptions were made in LS20 and the mutant derivatives were transformed with p42IRURA and p42IRLEU or with pDIR001. The following plasmids were used to disrupt genes in LS20: pDB121 (*rad51::KanMX*; this study), pKJ1112-S (*mre11::hisG-URA3-hisG*; Johzuka and Ogawa 1995), and pJH182 (*rad52::TRP1*; Moore and Haber 1996). The *RAD1* gene was disrupted with a KanMX PCR fragment (Wach *et al.* 1994). The *RAD51*, *RAD52*, and *MRE11* gene disruptions were confirmed phenotypically by sensitivity to 0.02% methyl methanesulfonate. The *RAD1* gene disruption was confirmed by sensitivity to ultraviolet light.

***GalHO* gene inductions:** The plate induction of the HO endonuclease gene was carried out as follows. Cells were grown at 30° in selective liquid medium with glucose [yeast complete (YC), −ura, 2% glucose; YC, −leu, 2% glucose; or YC, −ura, −leu, 2% glucose] to midlog phase. Cells were harvested by centrifugation, washed twice with sterile distilled water, and resuspended in the appropriate selective gylcerol medium (YC, −ura, 2% glycerol; YC, −leu, 2% glycerol; or YC, −ura, −leu, 2% glycerol) and incubated for 20–24 hr at 30°. Cells were harvested from the glycerol medium by centrifugation, washed twice with water, and plated to YC, −ura, 2% galactose medium; YC, −leu, 2% galactose; or YC, −ura, −leu, 2% galactose and incubated at 30°. For most experiments, plating density was ~1000 cells per plate. To calculate the plating efficiency on galactose, a volume of cell suspension equal to that plated onto galactose medium was plated to the appropriate glucose medium. For the *rad1*Δ and *rad52*Δ strains, galactose plates received 100 times more cells than the glucose control plates received. Colonies on the galactose plates were counted after 4 or 5 days of growth at 30°. Colonies on the glucose plates were counted after 2 or 3 days of growth at 30°. Clearly separated colonies from galactose plates were picked at random and streaked to glucose plates for stock cultures. Reported plating efficiencies on galactose are based on the average of at least three separate plating experiments.

Genomic DNA isolations, gel electrophoresis, and Southern blotting: Cells from appropriate stock plates were transferred to liquid glucose medium, grown to stationary phase, and genomic DNA was prepared using the Wizard genomic DNA preparation kit (Promega, Madison, WI). With this procedure, the bulk of purified chromosomal DNA was between 30 and 50 kbp in length (data not shown). Undigested genomic DNA was fractionated on 0.6% agarose gels. Restriction-digested DNAs were fractionated with 0.8% or 2% agarose gels, depending on the expected size of the target DNA. All Southern transfers were to GeneScreen nylon membranes. The pBR322-derived probe was labeled with ^{32}P using the random priming method. The oligonucleotide probe was labeled with ^{32}P by using T4 polynucleotide kinase. All DNA modification enzymes were purchased from Promega and were used according to the manufacturer's recommendations.

RESULTS

Experimental system for studying large palindrome formation: We have developed a simple method for inducing the formation of large DNA palindromes from nonpalindromic precursor DNA in Saccharomyces (Butler *et al.* 1996). The precursor DNA consists of either a 6.5-kbp plasmid called p42IRURA or a 10.5-kbp plasmid called p42IRLEU. Each plasmid contains all of the *cis*-acting sequence elements needed to form a stable linear palindrome (see Figure 2). These include a short inverted repeat sequence that consists of 42-bp inverted repeats separated by a 28-bp nonpalindromic spacer. The 42-bp inverted repeat sequence is located 20 bp to the left of a recognition site for the yeast endonuclease HO (referred to as the "left" HO cut site). Adjacent to a second HO cut site (the right HO cut site) is a short tract of $C_{1-3}A$ repeats to promote new telomere formation after HO cleavage. Located between the two HO cut sites is a centromere (CEN4) to maintain the precursor plasmids at single-copy levels in most cells (Hieter *et al.* 1985). CEN4 is flanked by the two HO cut sites, so that palindromes formed from these plasmids are acentric instead of dicentric. Each plasmid also includes a yeast origin of replication (ARS1) and a selectable marker (either *URA3* or *LEU2*). The p42IRURA and p42IRLEU plasmids are identical in sequence except for the selectable marker. There are no chromosomal HO cut sites in the host strains used in this study. All host strains harbor a chromosomally integrated copy of the HO endonuclease gene fused to a galactose responsive promoter. The activity of the gal promoter is such that growth on glucose represses transcription, while growth on galactose induces transcription and leads to cleavage of the precursor plasmids. Palindrome formation is monitored by plating cells containing precursor plasmids to various selective galactose media (see materials and methods for details). In a typical experiment using p42IRURA as the precursor plasmid, ~90% of the cells that survive on galactose medium without uracil form a stable 11-kbp palindrome (this equates to an overall rate of palindrome formation of ~5% of cells plated). The structure of the palindrome was confirmed by extensive restriction mapping and sequencing. The remaining galactose survivors typically harbor a small circular molecule derived from p42IRURA or chromosomally integrated p42IRURA DNA (Butler *et al.* 1996).

Evidence for an intermolecular recombination mechanism of palindrome formation: Figure 1 depicts the intramolecular and intermolecular recombination models of palindrome formation. To test for intermolecular reactions in palindrome formation, we constructed a strain, called ATB996, that harbors both p42IRURA and p42IRLEU. If homologous recombination between short inverted repeat sequences on different precursor molecules is the primary mechanism of palindrome formation, then ATB996 should predominantly form heteropalindromes. In a heteropalindromic molecule, one arm of the palindrome is derived from p42IRURA and the other arm is derived from p42IRLEU. It is also possible that a hetero-

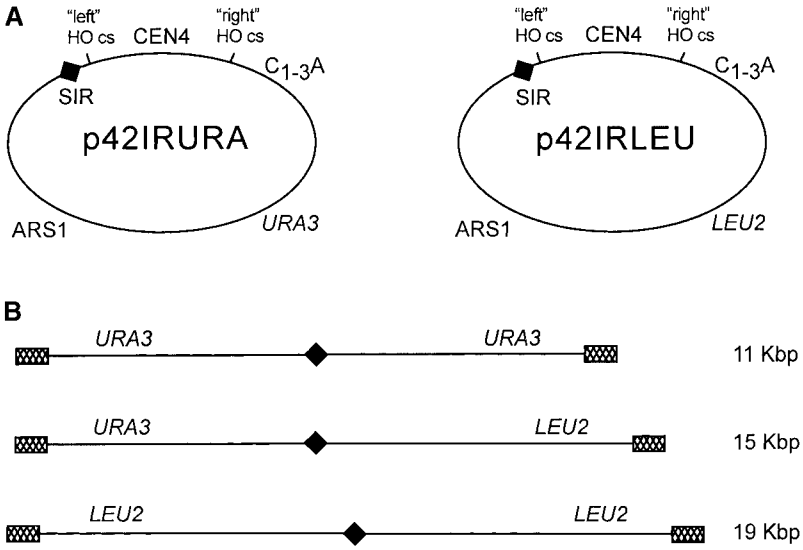


FIGURE 2.—(A) Schematic representation of the p42IRURA and p42IRLEU precursor plasmids. The 42-bp inverted repeat sequence is represented by the solid diamond. The two cleavage sites for the HO endonuclease are designated as "left" and "right" HOcs. $C_{1\text{-}3}A$ represents an ~80-bp tract of telomeric DNA sequence. ARS1 is a yeast origin of replication. CEN4 is a yeast centromere. The non-yeast DNA of these plasmids is derived from pBR322. The size of p42IRURA is ~6.5 kbp. The plasmid p42IRLEU is 10.5 kbp and is derived from p42IRURA by insertion of a 4.0-kbp fragment containing the *LEU2* gene into a unique *Nco*I site located in *URA3*. The hybridization probe used for the detection of palindromic DNA is derived from the pBR322 sequence located between the 42-bp inverted repeat sequence and ARS1. (B) Scheme of a URA-homopalindrome, a heteropalindrome, and a LEU-homopalindrome. Note that a single copy of the 42-bp inverted repeat sequence forms the center of each type of palindrome. The cross-hatched rectangle at the end of each palindrome represents a telomere. The predicted sizes of the palindromes are shown at the right of the diagram. The structure of the URA-homopalindrome has been confirmed by detailed restriction mapping and sequencing (BUTLER *et al.* 1996). The diagrams are not to scale.

palindromic molecule can form by a nonhomologous end joining (NHEJ) type of reaction. However, the structure of the center of a heteropalindrome formed by NHEJ would be different from that of a heteropalindrome formed by homologous intermolecular recombination (see below). If intramolecular recombination is the sole mechanism of palindrome formation, then this strain should form only homopalindromes (in a homopalindrome, both arms of the palindrome are derived from the same plasmid). The URA-homopalindrome is 11 kbp in length (BUTLER *et al.* 1996), while a heteropalindrome is expected to be 15 kbp in length and a LEU-homopalindrome is expected to be 19 kbp in length (Figure 2B).

Palindrome formation in ATB996 was analyzed by plating to galactose medium lacking only uracil, to galactose medium lacking only leucine, and to galactose medium lacking both uracil and leucine (prior to plating on the different galactose media, ATB996 is maintained on glucose medium that selects for both plasmids). The molecular structure of the precursor plasmid-derived DNA was analyzed in 52 colonies selected on galactose medium without uracil. For this analysis, undigested genomic DNA isolated from each colony was fractionated by electrophoresis with a 0.6% agarose gel and blotted, and the blot was hybridized with a probe specific to the p42IRURA and p42IRLEU plasmids. Out of 52 Ura^+ colonies analyzed, 16 harbored only the URA-homopalindrome (see lanes 3–6, 10, and 11 in Figure 3A, for example) and 36 colonies harbored a band migrating at ~15 kbp, the size expected for a heteropalindrome (see lanes 1, 2, and 7–9 in Figure 3A). Reprobing of the blot shown in Figure 3A with a *LEU2*-specific probe detected only the 15-kbp band, supporting the idea that the 15-kbp band is the result of heteropalindromic DNA (data not shown). Five of the Ura^+ colonies clearly harbored a mixture of URA-homopalindrome and heteropalindrome (see lanes 2 and 9 in Figure 3A, for example), suggesting that there can be more than one palindrome formation event in a colony-forming cell. Since the homopalindromes and heteropalindromes are acentric, random segregation during the first few cell divisions may result in differences in the amount of each type of palindrome during the outgrowth of a colony. The plating of ATB996 to galactose medium lacking leucine yielded analogous results. Approximately 38% of the Leu^+ colonies harbored a band of ~19 kbp (the size expected for a LEU-homopalindrome), while the remainder harbored the heteropalindrome band or a mixture of heteropalindrome and putative LEU-homopalindrome (data not shown). Finally, the structure of palindromic DNA was analyzed in 50 colonies selected on galactose medium without uracil and leucine. All 50 of the Ura^+ Leu^+ colonies analyzed harbored the heteropalindrome band (see Figure 3B for a representative analysis). None of the Ura^+ Leu^+ colonies harbored only homopalindromic DNA.

If, as expected, the heteropalindromes detected in the foregoing experiments are formed by homologous recombination of two short inverted repeat sequences, then these palindromes should have a single copy of the short inverted repeat sequence at their center. For such a palindrome, digestion with the restriction enzyme *Ban*II will release a central fragment of 266 bp, as a result of cutting 77 bp from either edge of the short inverted repeat sequence (the total size of the short inverted repeat element is 112 bp). A heteropalindrome formed by NHEJ would be expected to have a larger central *Ban*II fragment, since the central fragment would likely include two complete short inverted repeat sequences. Genomic DNA from several Ura^+ Leu^+ colonies was digested with

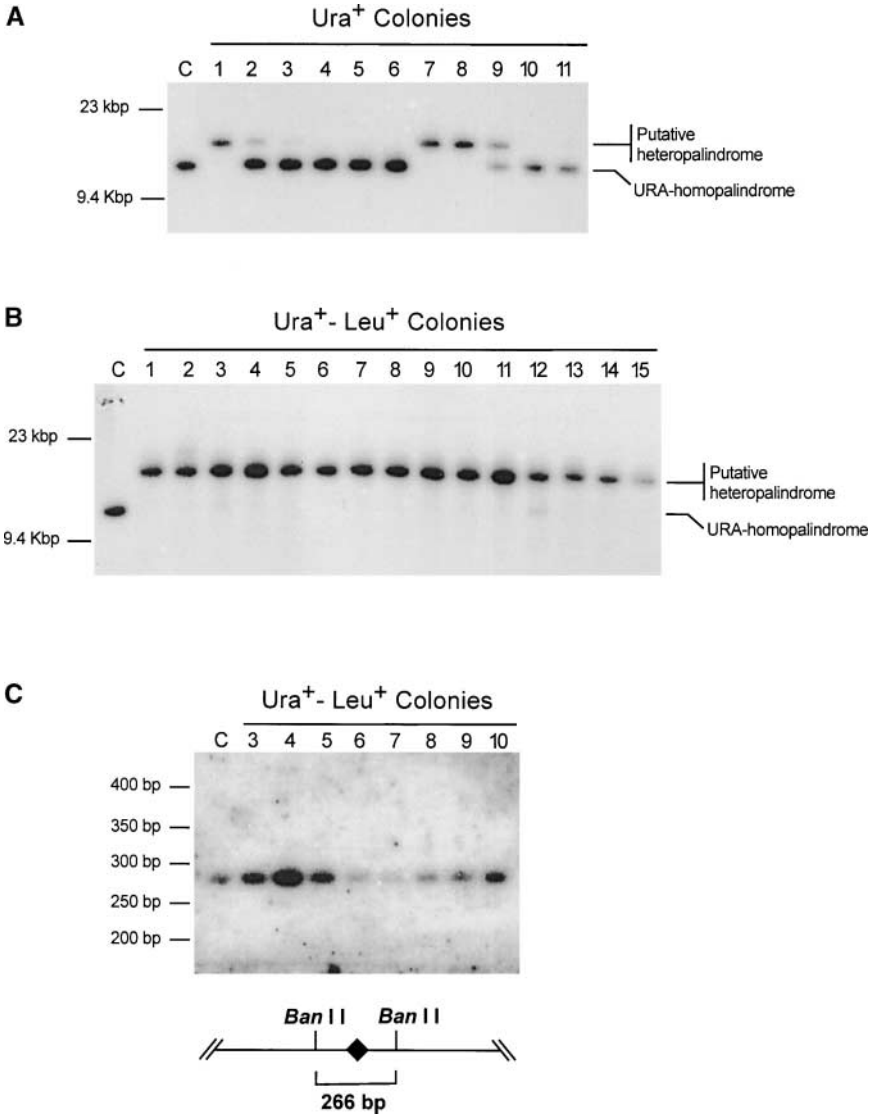
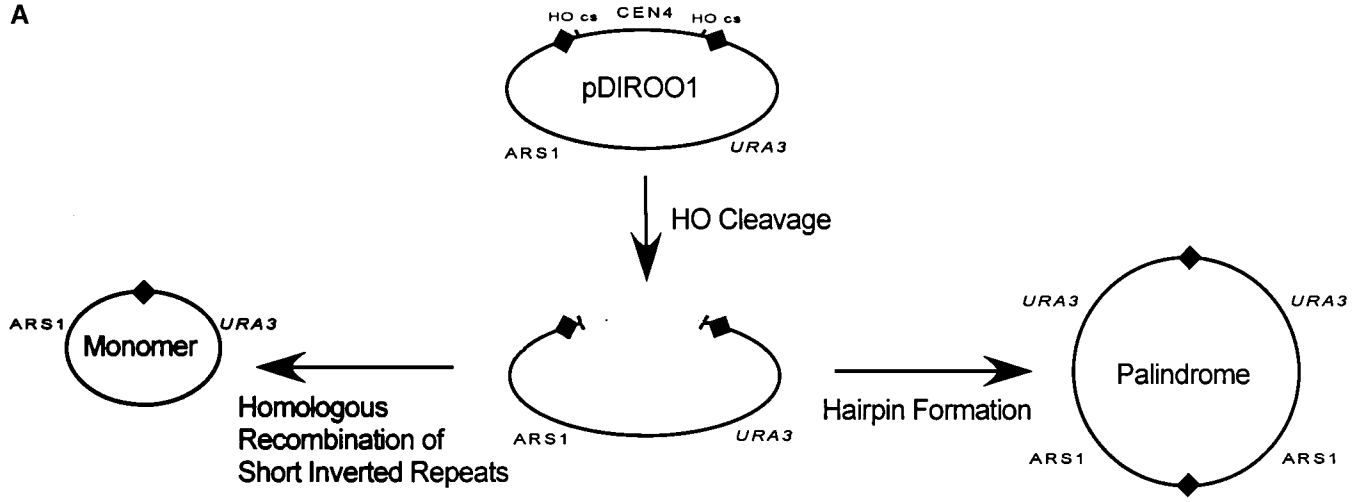


FIGURE 3.—Southern blot analysis of Ura$^+$ and Ura$^+$ Leu$^+$ colonies from the plating of Rad$^+$ ATB996 to selective galactose media. (A) Undigested genomic DNA from colonies isolated in minus-uracil medium was fractionated on a 0.6% agarose gel and blotted to a nylon membrane hybridized with the pBR322-derived probe complementary to all three types of palindromes. The positions of the URA-homopalindrome and putative heteropalindrome (referred to as the 15-kbp band in the main text) are indicated on the right. "C" designates undigested genomic DNA from a Rad$^+$ strain harboring a URA-homopalindrome. (B) Palindromes analyzed as in A. The positions of the URA-homopalindrome and putative heteropalindrome are indicated on the right. "C" designates undigested genomic DNA from a Rad$^+$ strain harboring a URA-homopalindrome. (C) *Ban*II analysis of selected putative heteropalindromes (the colony Ura$^+$ Leu$^+$ numbers correspond to those in B). Genomic DNA (~2 μg) from these colonies was digested with *Ban*II, fractionated on a 2.0% agarose gel, and blotted to a nylon membrane hybridized with an oligonucleotide probe complementary to the 28-bp nonpalindromic spacer of the short inverted repeat sequence. A scheme of the central region of a palindrome is shown. The short inverted repeat sequence is represented by a solid diamond. The symmetrically arranged *Ban*II recognition sites are also shown. The entire short inverted repeat sequence is 112 bp and each *Ban*II site is 77 bp from the edge of the short repeat sequence. "C" designates *Ban*II-digested genomic DNA from a Rad$^+$ strain harboring a URA-homopalindrome.

*Ban*II and subjected to Southern blot analysis using an oligonucleotide probe complementary to the nonpalindromic center of the short inverted repeat sequence. As can be seen in Figure 3C, the probe detects a band of ~270 bp in these colonies. Furthermore, this band was indistinguishable in size from the central *Ban*II fragment of a URA-homopalindrome whose center has been sequenced and is known to have a single copy of the short inverted repeat sequence (BUTLER *et al.* 1996). This central structure is consistent with the idea that the heteropalindromes were formed by homologous recombination of two short inverted repeat sequences and not by NHEJ.

Clearly, the formation of heteropalindromes is a common event when ATB996 cells are plated to galactose. This result provides direct support for an intermolecular recombination mechanism of palindrome formation. Interestingly, despite the apparent presence of two different precursor plasmids within the same cell, homopalindromes can also form readily in ATB996. An efficient intramolecular mechanism can explain the formation of the homopalindromes. However, it is also possible that the homopalindromes form by an intermolecular reaction in cells with more than one copy of a particular precursor plasmid. Multiple copies of a precursor plasmid can result from an occasional nondisjunction (*i.e.*, 2:0 segregation) event or when cells are in the G2 phase of the cell cycle.

When located near a double-strand break, two short inverted repeat sequences within the same plasmid efficiently participate in homologous recombination, but apparently not in hairpin formation: We developed a second assay to further address the potential reactions that the short inverted repeat sequence can undergo. For this assay we modified p42IRURA by replacing the telomeric sequence with an identical complete copy of the short inverted repeat sequence (42-bp inverted repeats separated by the 28-bp spacer). In this position, the second copy of the short inverted repeat sequence is located 20 bp to the right of the right HO cut site (see Figure 4A).



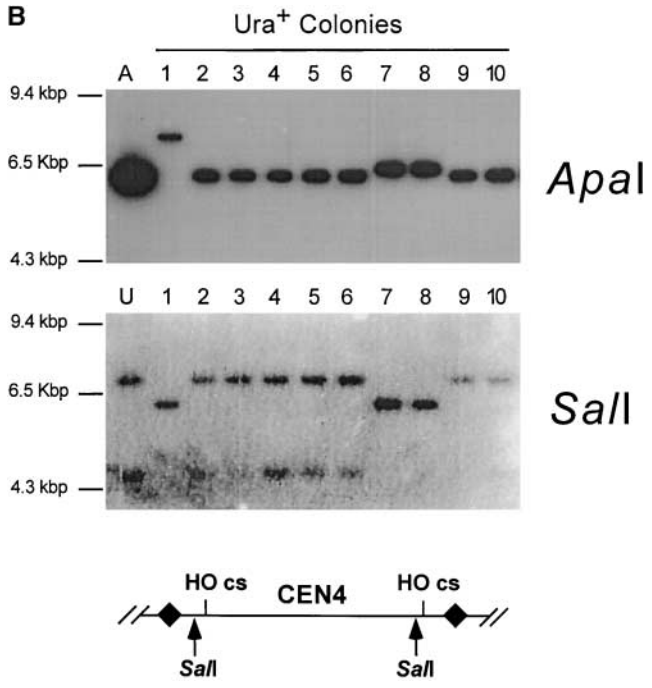


FIGURE 4.—Molecular analysis of repair events from cleaved pDIR001. (A) Scheme of intact pDIR001, HO-cleaved pDIR001 plasmid, and possible outcomes of short inverted repeat-mediated recombination reactions. The arrow pointing to the left represents a pathway involving homologous recombination of the two short inverted repeat sequences within pDIR001 to form a monomeric circular plasmid. This pathway mimics the intermolecular recombination reaction that occurs in the formation of heteropalindromes. The arrow pointing to the right represents a pathway involving short inverted repeat-mediated hairpin formation and bidirectional replication to form a palindromic circle. This pathway mimics the intramolecular recombination reaction diagrammed in Figure 1A to form homopalindromes. The diagrams are not to scale. (B) Southern blot analysis of Ura$^+$ colonies from plating Rad$^+$ ATB901 strain to selective galactose medium. Genomic DNA (~2 μg) from Ura$^+$ isolates was digested with either *Apa*I (top) or *Sal*I (bottom), fractionated on a 0.8% agarose gel, blotted to a nylon membrane, and hybridized with the pBR322 probe. "A" designates a control plasmid of the structure expected of a monomer digested with *Apa*I. "U" designates undigested control plasmid. The two bands visible in the undigested control lane and in lanes 2, 4, 5, and 6 of the *Sal*I gel panel are presumably relaxed and supercoiled forms of monomer. The bottom scheme is an expanded version of the CEN4 region of pDIR001 showing the location of *Sal*I sites. Two *Sal*I sites are in pDIR001: one is located between the "left" HO cs and the "left" short inverted repeat sequence, and the second is located ~10 bp on the CEN4 side of the "right" HO cs. A monomer formed by intermolecular recombination or a palindrome formed by intramolecular recombination will not retain either *Sal*I site. Any plasmid formed by NHEJ will most likely retain the "left" *Sal*I site. A pDIR001 plasmid that is not cleaved by HO will retain both original *Sal*I sites.

The key feature of this plasmid (called pDIR001) is that when it is cleaved at the HO cut sites, each end of the linear fragment will have an identical copy of a complete short inverted repeat sequence (Figure 4A). From cleaved pDIR001, we expect that the two terminal short inverted repeat sequences will be able to engage in homologous recombination. This reaction would mimic the type of intermolecular recombination reaction that must occur in the formation of heteropalindromes. In the present context, the outcome of this reaction would be the formation of a monomeric circle (Figure 4A). If, at some frequency, the short inverted repeat sequence is truly able to form a stable hairpin at a double-strand break, then the two ends of cleaved pDIR001, rather than recombining, would occasionally become capped by hairpins. Replication of such a double-hairpin intermediate would result in a palindromic circular plasmid. This reaction would mimic the type of intramolecular recombination reaction postulated in Figure 1A for the formation of homopalindromes. We are aware that a palindromic circle (as well as a direct repeat circle) could form by a bimolecular reaction. However, since this would involve recombination between short inverted repeat sequences on different molecules, we think that this will be a very inefficient reaction compared to recombination between short inverted repeat sequences in the same plasmid (*i.e.*, monomer

TABLE 1

Plating efficiency on selective galactose media and number of homopalindromes and heteropalindromes recovered in Rad$^+$ and Rad mutant strains

			Homo- and heteropalindrome formation[c]			
	Plating efficiency:[b] selection		Ura$^+$ selection		Ura$^+$ Leu$^+$ selection	
Strain[a]	% Ura$^+$	% Ura$^+$ Leu$^+$	Homo-	Hetero-	Homo-	Hetero-
Rad$^+$	5.0	3.8	16/52	36/52	0/50	50/50
*rad1*Δ	0.1	0.3	0/12	0/12	0/12	2/12
*rad51*Δ	8.0	5.8	6/12	6/12	0/12	12/12
*rad52*Δ	0.4	0.08	7/30	5/30	0/25	22/25
*mre11*Δ	6.6	1.1	23/36	6/36	0/18	17/18

[a] All strains are derivatives of ATB996.
[b] See MATERIALS AND METHODS for calculation of plating efficiency on galactose media.
[c] Only palindromes formed by homologous recombination of short inverted repeats are counted.

formation) and therefore not be a major source of palindrome formation.

We plated cells harboring the pDIR001 plasmid to galactose medium without uracil. The structure of pDIR001-derived DNA in 40 Ura$^+$ colonies was first analyzed by *Apa*I restriction digestion. In pDIR001 there is one recognition site for *Apa*I (located in the *URA3* gene). When a monomer derived from pDIR001 is digested with *Apa*I, a single 5.5-kbp fragment should result. Alternatively, a palindromic circle derived from pDIR001 is expected to have two *Apa*I sites. *Apa*I digestion of a palindromic circle will generate an 8.4-kbp fragment and a 2.6-kbp fragment (the probe used in this experiment will detect only the 8.4-kbp band). The *Apa*I analysis revealed three types of repaired plasmid among the Ura$^+$ colonies selected on galactose. For most of the colonies (33/40), the probe hybridized to a 5.5-kbp band (see Figure 4B, top, lanes 2–6, 9, and 10, for a representative example). Additional restriction analysis with *Sal*I confirmed that these Ura$^+$ colonies did in fact harbor monomeric plasmid formed by homologous recombination between the short inverted repeat sequences (Figure 4B, bottom, and see Figure 4 legend for the rationale of the *Sal*I analysis). In three colonies the probe hybridized to an *Apa*I band of ~8 kbp (see Figure 4B, top, lane 1, for an example). This band is close in size to that expected for a palindrome. However, *Sal*I digestion revealed that these colonies actually harbor intact pDIR001 that presumably was not cleaved by HO (Figure 4B, bottom). Three Ura$^+$ colonies harbored monomeric plasmid that resulted from NHEJ of HO cleaved ends (see Figure 4B, lanes 7 and 8) and one colony harbored chromosomally integrated pDIR001-derived DNA (data not shown). Thus, we did not recover any Ura$^+$ colonies with palindromic DNA. It is possible that palindromic circles can form from cleaved pDIR001, but that the circular palindromes are unstable and rapidly lost from the cell or become significantly rearranged. We do not think that this is likely, however, since stable palindromic circles have been detected previously in yeast (KUNES *et al.* 1984; HUANG and CAMPBELL 1995). We suggest, therefore, that the failure to detect palindromic circles is due to the inability of the short inverted repeat sequence to form a stable hairpin at a double-strand break.

Requirements for *RAD52*-epistasis group genes in palindrome formation: The *RAD52* gene is required for virtually all homologous recombination in Saccharomyces. *RAD52* is a member of a large epistasis group that includes the *RAD50*, *RAD51*, *RAD53*, *RAD54*, *RAD55*, *RAD57*, *RAD59*, *MRE11*, and *XRS2* genes (PAQUES and HABER 1999). In a previous study we found evidence that the *RAD52* gene may be important for palindrome formation. In Rad$^+$ cells, palindromes can be detected within 6–12 hr of *HO* gene induction. In contrast, in a *rad52*Δ mutant, palindromes could not be detected even at 48 hr postinduction, suggesting that *RAD52* is required for palindrome formation (BUTLER *et al.* 1996).

We wanted to extend this analysis using the galactose plate assay and a *rad52*Δ derivative of ATB996. When plated to galactose without uracil, the plating efficiency of *rad52*Δ ATB996 was 12-fold lower than that of Rad$^+$ ATB996 (0.4% *vs.* 5.0%; see MATERIALS AND METHODS for the calculation of plating efficiency on galactose). Among 30 Ura$^+$ colonies analyzed, only 7 harbored the URA-homopalindrome and 5 harbored heteropalindrome (Table 1). The remaining Ura$^+$ colonies contained a small extrachromosomal DNA molecule or chromosomally integrated plasmid DNA. When *rad52*Δ ATB996 was plated to galactose without both uracil and leucine, the plating efficiency dropped to 0.08%, 48-fold lower than that of the Rad$^+$ ATB996 strain (Table 1). Most of the Ura$^+$ Leu$^+$ colonies analyzed (22/25) harbored heteropalindromic DNA (Table 1). On the basis of *Ban*II analysis, the structure of the center of these heteropalindromes is consistent with homologous recombination between the short inverted repeats (data not shown). Nevertheless, it is clear that the overall frequency of homopalindrome and heteropalindrome formation is very low in the *rad52*Δ strain

compared to that of the Rad$^+$ strain. Thus, these results support the conclusion that the *RAD52* gene plays an important, if not strictly essential, role in palindrome formation by homologous recombination of short inverted repeat sequences.

To further explore the connection between palindrome formation and homologous recombination, we tested palindrome formation in a *rad51*Δ derivative of ATB996 and in a *mre11*Δ derivative of ATB996. The *RAD51* and *MRE11* genes were chosen for this analysis because they are thought to represent functionally important subdivisions within the *RAD52*-epistasis group (Paques and Haber 1999). The *RAD51* gene encodes a strand exchange protein that is similar to the bacterial RecA protein (*RAD55* and *RAD57* also encode RecA-like proteins). *MRE11* encodes a nuclease that is a member of the conserved Mre11p/Rad50p/Xrs2p complex. The complex is involved in processing the ends of DNA double-strand breaks for homologous recombination and NHEJ reactions (Haber 1998). Of particular relevance to this study, it has also been reported that the mre11 protein has hairpin nicking ability, raising the possibility that the Mre11/Rad50/Xrs2 complex may suppress the type of intramolecular pathway outlined in Figure 1A (Trujillo and Sung 2001). Deletion of *MRE11*, *RAD50*, or *XRS2* leads to an identical spectrum of phenotypes in yeast.

Deletion of the *RAD51* gene had no effect on the formation of palindromic DNA. The *rad51*Δ ATB996 strain displayed a plating efficiency on selective galactose media similar to that of the Rad$^+$ ATB996 strain and the molecular analysis of galactose survivors revealed that homopalindromes and heteropalindromes form readily in the *rad51*Δ ATB996 mutant (Table 1). Clearly palindrome formation by intermolecular recombination can operate independently of *RAD51*.

The galactose plating efficieny of the *mre11*Δ ATB996 strain was also similar to that of the Rad$^+$ strain (Table 1). Intriguingly, from the plating of *mre11*Δ ATB996 to galactose medium without uracil, there appeared to be a small (approximately two to threefold) excess of Ura$^+$ colonies with the URA-homopalindrome compared to when Rad$^+$ ATB996 was plated to the same medium (Table 1). This kind of result might be expected if the *MRE11* deletion allows the short inverted repeat sequence to form a stable hairpin at a double-strand break. However, an analysis of 18 Ura$^+$ Leu$^+$ colonies from plating *mre11*Δ ATB996 to galactose medium without uracil and leucine was inconsistent with the foregoing idea. None of the Ura$^+$ Leu$^+$ colonies analyzed harbored homopalindromic DNA, yet 17 out of the 18 colonies analyzed harbored heteropalindomic DNA (Table 1). Similarly, when we tested for the formation of circular palindromes from cleaved pDIR001 in a *mre11*Δ background, none of the 18 Ura$^+$ colonies analyzed harbored palindromic circles. All 18 colonies harbored monomeric plasmid formed by homologous recombination of the short inverted repeat sequences (data not shown). Thus, a deletion of *MRE11* does not appear to enable the short inverted repeat sequence to form a stable hairpin. We also conclude that palindrome formation by intermolecular recombination is not affected by the *mre11*Δ mutation.

The *RAD1* gene is critical for palindrome formation: A key feature of the recombination models of palindrome formation presented in Figure 1 is the removal of noncomplementary DNA located 3′ to the short inverted repeat sequence. This corresponds to 20 nucleotides of DNA derived from the region between the short inverted repeats and the left HO cut site. We know from an earlier study that this DNA is not present in a URA-homopalindrome (Butler *et al.* 1996). In Saccharomyces, a single-strand endonuclease, encoded by the *RAD1* and *RAD10* genes, is required to remove noncomplementary 3′ "tails" from so-called "flap" structures (Fishman-Lobell and Haber 1992; Bardwell *et al.* 1994; Ivanov and Haber 1995). The Rad1/Rad10 endonuclease presumably catalyzes the hydrolysis of the phosphodiester bond between the last paired nucleotide and the first unpaired nucleotide of the tail, thus making a fully homologous 3′ end. To address whether the Rad1/Rad10 endonuclease plays a role in the palindrome formation reaction, we analyzed palindrome formation in a *rad1*Δ ATB996 strain. The formation of homopalindromes and heteropalindromes is severely compromised by the deletion of *RAD1*. When plated to galactose without uracil, the plating efficiency of *rad1*Δ ATB996 was 14-fold lower than that of Rad$^+$ ATB996 (Table 1). Among 12 Ura$^+$ colonies analyzed, none harbored either the URA-homopalindrome or the heteropalindrome formed by homologous recombination of short inverted repeat sequences (Table 1). Various kinds of products were detected among the Ura$^+$ colonies, including a small extrachromosomal DNA molecule, chromosomally integrated plasmid DNA, and a palindrome formed by NHEJ (data not shown). When plated to galactose without uracil and leucine, the plating efficiency of *rad1*Δ ATB996 was only 12-fold lower than that of the Rad$^+$ ATB996 strain (Table 1). However, only 2 out 12 Ura$^+$ Leu$^+$ colonies harbored heteropalindromic DNA (Table 1). On the basis of *Ban*II analysis, the structure of the center of these heteropalindromes is consistent with their having been formed by homologous recombination of short inverted repeat sequences (data not shown). We conclude that the Rad1/Rad10 endonuclease is critical for efficient palindrome formation by intermolecular recombination of short inverted repeats.

DISCUSSION

In this study we have presented evidence that directly supports an intermolecular mechanism of palindrome formation in budding yeast. We have used a strain bearing two genetically and physically marked precursor plasmids to show that the two arms of a palindrome are

frequently derived from different precursor molecules. The formation of such heteropalindromes is best explained by an intermolecular reaction. The structure at the center of the heteropalindromes is consistent with the idea that homologous recombination between two short inverted repeat sequences brings together the precursor fragments in a head-to-head arrangement. A homologous recombination-based mechanism is further supported by the finding that palindrome formation is strongly dependent on the *RAD1* and *RAD52* genes. Under certain selective conditions we also recovered palindromes formed from only one type of precursor plasmid. These homopalindromes could have been formed by an intramolecular recombination reaction, that is, by a reaction involving hairpin formation mediated by the short inverted repeat sequence. However, the experiments with pDIR001 imply that hairpin formation with the short inverted repeat sequence is at best rare. Thus, we favor the idea that the homopalindromes are also formed by an intermolecular reaction involving homologous recombination of short inverted repeat sequences.

The physical and genetic analysis presented above clearly establishes that, in our system, homologous recombination is the underlying molecular basis of palindrome formation. In Saccharomyces, at least three major pathways of homologous recombination can be triggered by a DNA double-strand break: gene conversion with or without crossing over (these two outcomes are mechanistically related by strand invasion and Holliday junction formation), single-strand annealing, and break-induced replication (see Haber 2000 for review). In principle, each of these recombination pathways has the potential to account for an intermolecular pathway of palindrome formation. Under the conditions of our experiments, we tend to favor single-strand annealing (SSA) as the primary homologous recombination pathway of palindrome formation (Figure 1B represents the SSA version of palindrome formation). The genetic requirements for palindrome formation are similar to those of SSA recombination. SSA recombination involving small repeats is largely *RAD52* dependent and *RAD51* independent (Ivanov *et al.* 1996; Sugawara *et al.* 2000). In contrast, recombination reactions involving Holliday junctions generally require the *RAD51* gene, as well as most other members of the *RAD52* epistasis group (Ivanov *et al.* 1996), and break-induced replication, though it is *RAD51* independent, apparently requires the *MRE11* gene (Malkova *et al.* 1996; Signon *et al.* 2001). We acknowledge, however, that non-SSA pathways of recombination may prevail under genetic or genomic conditions different from those simulated in this study.

Large palindromes have been observed under a variety of circumstances in Saccharomyces. Recently it was discovered that an ~700-kbp palindrome derived from chromosome II forms readily in *mre11*Δ, *rad50*Δ, *xrs2*Δ, or *sae2*Δ mutants, but only very inefficiently in Rad$^+$ cells (Lobachev *et al.* 2002). A key event in the formation of this palindrome involves a spontaneous DNA double-strand break within an inverted repeat composed of human Alu elements (each repeat is 320 bp in length and the repeats are separated by a 12-bp spacer). Apparently the Alu inverted repeat is unstable and is efficiently extruded as a cruciform that becomes the target of a structure-specific endonuclease. In cells lacking the Mre11/Rad50/Xrs2 complex or the Sae2 protein, the Alu inverted repeat is able to form a stable hairpin at the double-strand break (presumably these mutants cannot destroy hairpins). Subsequent DNA replication then forms the giant palindromic chromosome. Even in a *mre11*Δ mutant background, we found no compelling evidence to indicate that the short inverted repeat sequence used in this study forms a stable hairpin at a double-strand break. We suspect that this is due to the small size of the repeats, as well as the spacing between the repeats. In support of this idea it has been shown that the recombinogenic potential of inverted repeats, which is thought to reflect their potential to form cruciforms or hairpins, declines with decreasing repeat size and increasing spacer size (Ruskin and Fink 1993; Lobachev *et al.* 1998).

Large palindromes have also been detected following transformation of linearized plasmid molecules lacking homology to the yeast genome (Kunes *et al.* 1984, 1990). In this particular case, the palindromes were exclusively circular in structure. We did not detect circular palindromes in any of our experiments. Interestingly, the circular palindromes detected by Kunes *et al.* (1984, 1990) were clearly formed from two precursor molecules in an intermolecular reaction. Sequence analysis has revealed that the two segments of the circular palindromes were often joined at ends bearing from 2 to 6 bp of homology (Kunes *et al.* 1986). This may appear superficially similar to the process we have described in this study; however, joining broken ends at microhomologies is more indicative of NHEJ, a pathway that functions independently of *RAD1* and *RAD52* (Moore and Haber 1996). Establishing the genetic requirements for the formation of the circular palindromes would help clarify their mechanistic origin. Large palindromes have also been observed following selection for amplification of the *ADH4* and *DFR1* genes (Walton *et al.* 1986; Dorsey *et al.* 1992; Huang and Campbell 1995; Moore *et al.* 2000) and as one class of products resulting from a double-strand break (DSB) within an inverted repeat substrate (Rattray *et al.* 2001). In the latter case, break-induced replication was postulated to form two precursor molecules that subsequently fused in a head-to-head arrangement by NHEJ.

Can intermolecular recombination of short inverted repeat sequences be a general pathway of palindrome formation? In our system we were able to supply cells with the appropriate precursor molecules for palindrome formation—two identical DNA fragments, each with a copy of the short inverted repeat sequence near a bro-

ken end—by transformation of *in vitro* constructed plasmids and the *in vivo* expression of a site-specific endonuclease. Obviously, this is not a situation likely to be experienced by most cells. In thinking about the possible general nature of an intermolecular recombination pathway, an important issue is how the appropriate precursor molecules can be generated during normal cell growth. Of course, all cell types will produce two potential precursor molecules in the form of sister chromatids with every round of DNA replication. Two DSBs, each occurring near identical short inverted repeat sequences on sister chromatids, would produce the precursor molecules needed for palindrome formation. It is extremely unlikely, however, that DSBs, which generally arise spontaneously and at random in a genome, will occur at the right position on two chromatids. A more plausible scenario could begin with a single DSB occurring near a short inverted repeat sequence in an unreplicated chromosome. Replication of the broken chromosome (before 5′ to 3′ resectioning reaches the short inverted repeat sequence) would generate two DNA fragments, each with a copy of the short inverted repeat near a broken end. Recombination between the terminal short inverted repeat sequences before the ensuing cell division would form the large palindrome (the large palindrome would be either acentric or dicentric, depending on whether the initial DSB was centromere proximal or centromere distal to the short inverted repeat). Alternatively, a single broken chromosome with a terminal short inverted repeat sequence could invade an intact sister chromatid or an intact homolog at a complementary short inverted repeat sequence and initiate break-induced replication to form a palindromic chromosome. Given the ubiquity of homologous recombination pathways and the common occurrence of short inverted repeat sequences in prokaryotic and eukaryotic genomes (see Bachellier *et al.* 1999 and *C. elegans* Sequencing Consortium 1998, for example), we think that intermolecular recombination of short inverted repeat sequences has the potential to be a widespread pathway of large DNA palindrome formation.

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LITERATURE CITED

Albrecht, E. B., A. B. Hunyady, G. R. Stark and T. E. Patterson, 2000 Mechanism of sod2 gene amplification in *Schizosaccharomyces pombe*. Mol. Biol. Cell **11:** 873–886.

Bachellier, S., J.-M. Clement and M. Hofnung, 1999 Short palindromic repetitive DNA elements in enterobacteria: a survey. Res. Microbiol. **150:** 627–639.

Bardwell, J. A., L. Bardwell, A. E. Tomkinson and E. C. Friedberg, 1994 Specific cleavage of model recombination and repair intermediates by the yeast Rad1-Rad10 DNA endonuclease. Science **265:** 2082–2085.

Bi, X., and L. F. Lui, 1996 DNA rearrangement mediated by inverted repeats. Proc. Natl. Acad. Sci. USA **93:** 819–823.

Butler, D. K., L. E. Yasuda and M.-C. Yao, 1995 An intramolecular recombination mechanism for the formation of the rRNA gene palindrome of *Tetrahymena thermophila*. Mol. Cell. Biol. **15:** 7117–7126.

Butler, D. K., L. E. Yasuda and M.-C. Yao, 1996 Induction of large palindrome formation in yeast: implications for gene amplification and genome stability. Cell **87:** 1115–1122.

C. elegans Sequencing Consortium, 1998 Genome sequence of nematode C. elegans: a platform for investigating biology. Science **282:** 2012–2018.

Dorsey, M., C. Peterson, K. Bray and C. E. Paquin, 1992 Spontaneous amplification of the *ADH4* gene in *Saccharomyces cerevisiae*. Genetics **132:** 943–950.

Fishman-Lobell, J., and J. Haber, 1992 Removal of nonhomologous ends in double-strand break recombination: the role of the yeast ultraviolet repair gene *RAD1*. Science **258:** 480–484.

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

Ford, M., B. Davies, M. Griffiths, J. Wilson and M. Fried, 1985 Isolation of a gene enhancer within an amplified inverted duplication after "expression selection." Proc. Natl. Acad. Sci. USA **82:** 3370–3374.

Fried, M., S. Feo and E. Heard, 1991 The role of inverted duplication in the generation of gene amplification in mammalian cells. Biochim. Biophys. Acta **1090:** 143–155.

Haber, J. E., 1998 The many interfaces of Mre11. Cell **95:** 583–586.

Haber, J. E., 2000 Partners and pathways: repairing a double-strand break. Trends Genet. **16:** 259–264.

Hieter, P., C. Mann, M. Snyder and R. W. Davis, 1985 Mitotic stability of yeast chromosomes: a colony color assay that measures nondisjunction and chromosome loss. Cell **40:** 381–392.

Huang, T., and J. L. Campbell, 1995 Amplification of a circular episome carrying an inverted repeat of DFR1 locus and adjacent autonomously replicating sequence element of Saccharomyces cerevisiae. J. Biol. Chem. **270:** 9607–9614.

Hyrien, O., M. Debatisse, G. Buttin and R. De Saint Vincent, 1988 The multicopy appearance of a large inverted duplication and the sequence at the inversion joint suggest a new model for gene amplification. EMBO J. **7:** 407–417.

Ivanov, E. L., and J. Haber, 1995 *RAD1* and *RAD10*, but not other excision repair genes, are required for double-strand break induced recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **15:** 2245–2251.

Ivanov, E. L., N. Sugawara, J. Fishman-Lobell and J. Haber, 1996 Genetic requirements for the single-strand annealing pathway of double-strand break repair in *Saccharomyces cerevisiae*. Genetics **142:** 693–704.

Johzuka, K., and H. Ogawa, 1995 Interaction of Mre11 and Rad50: two proteins required for DNA repair and meiosis-specific double strand break formation in *Saccharomyces cerevisiae*. Genetics **139:** 1521–1532.

Kunes, S., D. Botstein and M. S. Fox, 1984 Formation of inverted dimer plasmids after transformation of yeast with linearized plasmid DNA. Cold Spring Harbor Symp. Quant. Biol. **49:** 617–628.

Kunes, S., D. Botstein and M. S. Fox, 1986 Synapsis-dependent illegitimate recombination in yeast. Curr. Comm. Mol. Biol. **9:** 149–156.

Kunes, S., D. Botstein and M. S. Fox, 1990 Synapsis-mediated fusion of free DNA ends forms inverted dimer plasmids in yeast. Genetics **124:** 67–80.

Lin, F.-L., K. Sperle and N. Sternberg, 1984 Model for homologous recombination during transfer of DNA into mouse L cells: role for DNA ends in the recombination process. Mol. Cell. Biol. **4:** 1020–1034.

Lobachev, K., B. M. Shor, H. T. Tran, W. Taylor, J. D. Keen *et al.*, 1998 Factors affecting inverted repeat stimulation of recombination and deletion in *Saccharomyces cerevisiae*. Genetics **148:** 1507–1524.

Lobachev, K., D. A. Gordenin and M. A. Resnick, 2002 The Mre11 complex is required for repair of hairpin-capped double-strand breaks and prevention of chromosome rearrangements. Cell **108:** 183–193.

Malkova, A., E. L. Ivanov and J. E. Haber, 1996 Double-strand break repair in the absence of RAD51 in yeast: a possible role for break-induced DNA replication. Proc. Natl. Acad. Sci. USA **93:** 7131–7136.

Moore, J. K., and J. E. Haber, 1996 Cell cycle requirements of two pathways of nonhomologous end-joining of double-strand breaks in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:** 2164–2173.

Moore, I. K., M. P. Martin and C. E. Paquin, 2000 Telomere sequences at the novel joints of four independent amplifications in *Saccharomyces cerevisiae*. Environ. Mol. Mutagen. **36:** 105–112.

Nalbantoglu, J., and M. Mueth, 1986 DNA amplification-deletion in a spontaneous mutation of the hamster *aprt* locus: structure and sequence of the novel joint. Nucleic Acids Res. **14:** 8361–8371.

Ouellette, M., E. Hettema, D. Wust, F. Fase-Fowler and P. Borst, 1991 Direct and inverted DNA repeats associated with P-glycoprotein gene amplification in drug resistant *Leishmania*. EMBO J. **10:** 1009–1016.

Paques, F., and J. E. Haber, 1999 Multiple pathways of recombination induced by double strand breaks in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **63:** 349–404.

Qin, Z., and S. N. Cohen, 2000 Long palindromes formed in Streptomyces by nonrecombinational intra-strand pairing. Genes Dev. **14:** 1789–1796.

Rattray, A. J., C. B. McGill, B. K. Schafer and J. N. Strathern, 2001 Fidelity of mitotic double-strand-break repair in *Saccharomyces cerevisiae*: a role for *SAE2/COM1*. Genetics **158:** 109–122.

Rayko, E., 1997 Organization, generation and replication of amphimeric genomes: review. Gene **199:** 1–18.

Ruskin, B., and G. Fink, 1993 Mutations in *POL1* increase mitotic instability of tandem inverted repeats in *Saccharomyces cerevisiae*. Genetics **133:** 43–56.

Sandell, L. L., and V. A. Zakian, 1993 Loss of a yeast telomere: arrest, recovery and chromosome loss. Cell **75:** 729–739.

Signon, L., A. Malkova, M. L. Naylor, H. Klein and J. E. Haber, 2001 Genetic requirements for *RAD51*- and *RAD54*-independent break-induced replication repair of a chromosomal double-strand break. Mol. Cell. Biol. **21:** 2048–2056.

Sugawara, N., G. Ira and J. E. Haber, 2000 DNA length dependence of the single-strand annealing pathway and the role of *Saccharomyces cerevisiae RAD59* in double-strand break repair. Mol. Cell. Biol. **20:** 5300–5309.

Trujillo, K. M., and P. Sung, 2001 DNA structure-specific nuclease activities in the *Saccharomyces cerevisiae* Rad50-Mre11 complex. J. Biol. Chem. **276:** 35458–35464.

Wach, A., A. Brachat, R. Pohlmann and P. Philippsen, 1994 New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. Yeast **10:** 1793–1808.

Walton, J. D., C. E. Paquin, K. Kaneko and V. M. Williamson, 1986 Resistance to antimycin A in yeast by amplification of *ADH4* on a linear, 42 kb palindromic plasmid. Cell **46:** 857–863.

Yao, M.-C., S.-G. Zhu and C.-H. Yao, 1985 Gene amplification in Tetrahymena thermophila: formation of extrachromosomal palindromic genes coding for rRNA. Mol. Cell. Biol. **5:** 1260–1267.

Yasuda, L. F., and M.-C. Yao, 1991 Short inverted repeats at a free end signal large palindromic DNA formation in Tetrahymena. Cell **67:** 505–516.

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