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Characterization of a Centromere-Linked Recombination Hot Spot in *Saccharomyces cerevisiae*

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A 1.5-kilobase-pair *SalI-Hin*dIII (SH) restriction fragment from the region of *Saccharomyces cerevisiae* chromosome XIV immediately adjacent to the centromere appears to contain sequences that act as a hot spot for mitotic recombination. The presence of SH DNA on an autonomously replicating plasmid stimulates homologous genetic exchange between yeast genomic sequences and those present on the plasmid. In all recombinants characterized, exchange occurs in plasmid yeast sequences adjacent to rather than within the SH DNA. Hybridization analyses reveal that SH-containing plasmids are present in linear as well as circular form in *S. cerevisiae* and that linear forms are generated by cleavage at specific sites. Presumably, it is the linear form of the plasmid that is responsible for the stimulation of genetic exchange. Based on these observations, it is proposed that this DNA fragment contains a centromere-linked recombination hot spot and that SH-stimulated recombination occurs via a mechanism similar to double-strand-gap repair (J. W. Szostak, T. Orr-Weaver, J. Rothstein, and F. Stahl, Cell 33:25–35 1983).

Recombination hot spots are DNA sequences that stimulate genetic exchange in adjoining DNA segments; such hot spots have been identified in both procaryotes and eucaryotes. Several meiotic recombination hot spots have been identified, including the M26 mutation of *ade6* in *Schizosaccharomyces pombe* (8), the YS17 mutation of the *buff* spore color locus in *Sordaria brevicolis* (18), and the *cog*$^+$ mutation in *Neurospora crassa* (1). In addition, the *HOT1* gene in the rRNA gene cluster in *Saccharomyces cerevisiae* functions in *cis* to stimulate mitotic recombination (13). Available evidence suggests that these elements function by generating recognition sites for recombination endonucleases or by increasing the accessibility of flanking sequences to recombination enzymes.

Recently, it has been postulated that in *S. cerevisiae* some meiotic and mitotic recombination events occur via a double-strand-gap repair mechanism (33). According to this model, double-strand breaks are introduced into DNA and then enlarged to double-strand gaps. Crossing over and gene conversion occur during gap repair.

The general phenomenon of crossing over in the region of centromeres (*CEN*) has been of particular interest to geneticists because distances between markers in these regions are often disparate when cytological and genetic maps are compared (21, 27). In addition, mitotic and meiotic maps often disagree owing to the fact that the proportion of mitotic exchange events in some *CEN*-proximal regions is much higher than the relative frequency of meiotic exchange events in these regions (19, 21, 27). There are several explanations which either independently or together could account for this phenomenon. For example, centromeres repress meiotic crossing over in adjoining regions. This has been demonstrated to be true for the centromere on chromosome III (*CEN3*) of *S. cerevisiae* in an experiment in which wild-type *CEN3* sequences were deleted and integrated in a new location on one arm of chromosome III (17). Another as yet untested hypothesis is that an additional effect of centromeres is to stimulate mitotic recombination in adjacent DNA. Finally, there may be genetic elements that stimulate mitotic exchange in the vicinity of some centromeres. The results of experiments described here suggest that such an element occurs immediately adjacent to *CEN14* of *S. cerevisiae*.

In this paper, we present a characterization of a mitotic recombination-enhancing activity contained within a 1.5-kilobase-pair (kb) *Sal*I-*Hin*dIII restriction fragment (SH fragment) originating from the centromere region of yeast chromosome XIV. The presence of SH DNA on YRp7 plasmids stimulates homologous genetic exchange between yeast genomic sequences and those present on the plasmid. In all recombinants characterized, exchange occurred in yeast sequences present on the plasmid adjacent to the SH DNA. Hybridization analyses revealed that SH-containing plasmids are present in both linear and circular forms in yeasts and that linear forms are generated by cleavage at specific sites. Control plasmids that lack SH DNA are also present as circular and linear forms in *S. cerevisiae*; however, they appear to be linearized by cleavage at random sites. Based on these observations, it is proposed that (i) sequences contained within the SH fragment provide hot spot function and that (ii) SH-stimulated recombination occurs via a mechanism similar to double-strand-gap repair.

MATERIALS AND METHODS

Strains, media, and enzymes. *Escherichia coli* JA300 (*thrA leuB6 hsdR hsdM trpC117 strR*) was used for transformations and preparation of plasmid DNAs. *S. cerevisiae* strains used in this study were J17 (α*his2 ura3 ade1 met14 trp1*), RH218 (**a** *trp1 gal1 mal SUC2 CUP2*), SB61383-1C (**a** *trp1 ura3-52 ade1 met14 arg5-6*), and SB7883-1C (**a**/α *ura3-52/ura3-52 trp1/trp1 leu2-3/LEU2-3 his4/HIS4 cry1/CRY1 MET14/met14 ADE1/ade1*). Yeast strains RH218 (22), J17 (6), SB61383-1C (5), and SB7883-1C (5) have been described previously. Yeast matings, sporulations, and dissections were done as described previously (36). Media for yeast and bacterial growth have been described elsewhere (12). Restriction endonucleases and T4 DNA ligase were from New England BioLabs, Inc. (Beverly, Mass.). Buffer and reaction conditions were those specified by the vendor.

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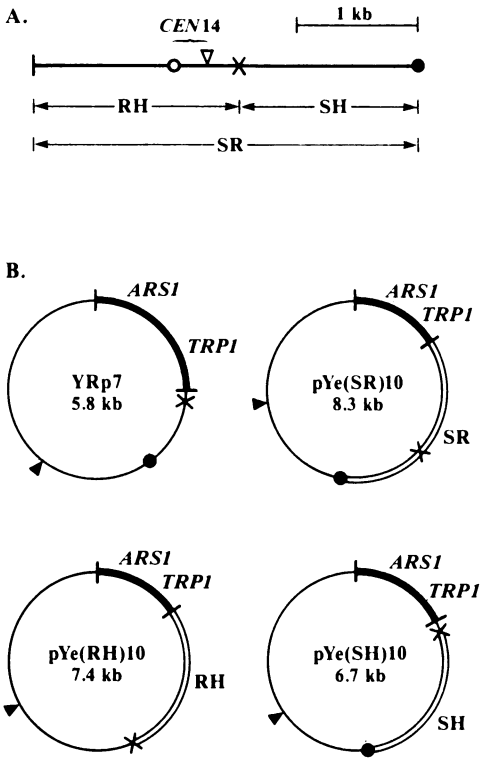

FIG. 1. (A) Restriction map and subcloning strategy of the 3.1-kb *Sal*I-*Eco*RI restriction fragment containing *CEN14*. (B) Plasmids constructed by subcloning the indicated restriction fragments into vector plasmid YRp7. See Materials and Methods for details of the plasmid constructions. These plasmids are not drawn to the same scale. The restriction sites are as follows: +, *Eco*RI; X, *Hin*dIII; ○, *Rsa*I; ●, *Sal*I; ▽, *Eco*RV; ▼, *Pvu*II. Thin lines represent pBR322 DNA, thick lines indicate *TRP1 ARS1* sequences, and the open bars represent a fragment from the *CEN* region of yeast chromosome XIV.

Plasmid constructions. Plasmid pCH10 has been described previously (12, 24). This plasmid was isolated from a yeast genomic library constructed by inserting *Sau*3A partial yeast genomic fragments into the *Bam*HI site of plasmid YRp7 (30). Plasmid pCH10 contains an approximately 10-kb yeast DNA insert which includes a 3.1-kb *Sal*I-*Eco*RI fragment that contains the centromere and flanking sequences from yeast chromosome XIV (Fig. 1). The 3.1-kb fragment was previously shown to be contiguous in the yeast genome (24).

Plasmid pCH10d was derived from plasmid pCH10 by removing a *Sal*I restriction fragment that includes 3.5 kb of the yeast insert DNA and the adjacent 0.3-kb fragment of pBR322 between the *Bam*HI and *Sal*I sites. The pertinent features of plasmid pCH10d are that it is a YRp7-type plasmid and that the yeast DNA insert includes the 3.1-kb *CEN14*-containing *Sal*I-*Eco*RI fragment (Fig. 1A).

The structures of plasmids pYe(SR)10, pYe(RH)10, and pYe(SH)10 are shown schematically in Fig. 1. Briefly, the 3.1-kb *Sal*I-*Eco*RI fragment containing *CEN14* and flanking DNA was ligated into plasmid pBR322 to replace the 0.65-kb *Sal*I-*Eco*RI vector fragment. The 1.5-kb *Eco*RI fragment containing the yeast *TRP1 ARS1* sequences was then inserted into the *Eco*RI site of the resultant plasmid to produce plasmid pYe(SR)10. Similarly, to generate plasmid pYe(RH)10, the *Eco*RI-*Hin*dIII fragment containing *CEN14* was ligated in place of the small *Eco*RI-*Hin*dIII fragment of pBR322 to form plasmid pBR-RH. The 1.5-kb *TRP1 ARS1*-containing fragment was inserted into the *Eco*RI site of plasmid pBR-RH to form plasmid pYe(RH)10. Likewise, plasmids pYe(SH)10 and pYe(SH)10′ were constructed by first inserting the 1.5-kb *Sal*I-*Hin*dIII *CEN14*-adjacent fragment (Fig. 1) in place of the 0.63-kb fragment of plasmid pBR322 to make plasmid pBR-SH. The 1.5-kb *TRP1 ARS1* fragment was inserted in the *Eco*RI site of plasmid pBR-SH in both orientations to give rise to plasmids pYe(SH)10 and pYe(SH)10′ (see Fig. 5).

Plasmids pYe(ESH)10 and pYe(ESH)10′ were made by first ligating the SH fragment into the *Sal*I-*Hin*dIII site in the polylinker of plasmid pUC12 (obtained from Bethesda Research Laboratories, Inc., Gaithersburg, Md.). A 1.5-kb *Eco*RI-*Hin*dIII fragment containing the SH DNA plus 30 base pairs of the polylinker was isolated from this construct and inserted in place of the small *Eco*RI-*Hin*dIII fragment of pBR322. The 1.5-kb *Eco*RI fragment containing the *TRP1 ARS1* region was inserted in both orientations in the *Eco*RI site of the resultant plasmid, thus generating plasmids pYe(ESH)10 and pYe(ESH)10′ (see Fig. 5).

Plasmid pMN105 was constructed by using plasmid pBR-SH (see above) as the vector and cloning into the *Hin*dIII site a 2.9-kb *Hin*dIII fragment containing *CEN3*. This *Hin*dIII fragment was isolated from plasmid pYe(CDC10)1 (7) and contains a 2.6-kb DNA fragment derived from the centromere region of yeast chromosome III and a 0.3-kb fragment derived from the region of plasmid pBR322 extending from the *Eco*RI site to the *Bam*HI site. Since most of this 0.3-kb region of pBR322 DNA was removed in constructing plasmid pBR-SH, only the 30-base-pair *Eco*RI-*Hin*dIII fragment of pBR322 is present in duplicate in plasmid pMN105. Plasmid pMN105 also contains the 1.5-kb *TRP1 ARS1* sequences inserted in the *Eco*RI site.

Plasmid pMN104 was made by using plasmid pBR-RH (see above) as the vector into which was inserted a 2.0-kb *Bam*HI-*Hin*dIII fragment containing *CEN3* and flanking sequences as well as the 0.3-kb *Eco*RI-*Bam*HI fragment of pBR322 and the 1.5-kb *Eco*RI *TRP1 ARS1* fragment. The 2.0-kb *CEN3*-containing fragment was derived from plasmid pYe(CDC10)1 (7). There are no duplicated sequences in plasmid pMN104.

DNA preparations and transformations. Purified plasmid DNA was isolated from *E. coli* as described by Holmes and Quigley (10). Yeast genomic DNA was prepared as described by Clarke and Carbon (5), and yeast plasmid DNA was prepared as described by Tschumper and Carbon (36). Yeast and *E. coli* transformations were described previously (11, 35).

Southern analysis, direct hybridization in agarose gels, and colony hybridization. Hybridization analyses were used to examine yeast cells for the presence of recombinant plasmids. *S. cerevisiae* colony hybridizations were as described previously (36). Southern analysis was done as described by Southern (29). Direct hybridization analysis of DNA in agarose gels was done as described by Tsao et al. (34). DNA probes were labeled by nick translation as described by Rigby et al. (28).

OFAGE. Orthogonal field alternation gel electrophoresis (OFAGE) analysis was used to identify yeast chromosomes involved in recombination with plasmid pYe(SH)10. Yeast chromosomes were prepared as described previously (M. Neitz, Ph.D. thesis, University of California, Santa Barbara, 1986). Running conditions were essentially those described by Carle and Olson (4) with modifications described in the legends to Fig. 4 and 6.

Patch test for plasmid recombination. A patch test was done to determine whether SH-stimulated recombination depends on the relative orientation in the plasmid of the SH DNA fragment and the 1.5-kb *Eco*RI restriction fragment containing the yeast *TRP1* gene and the replicator, *ARS1*. Single colonies were grown under conditions selecting for the plasmid genetic marker, *TRP1*, and then streaked in patches onto nonselective agar plates. After growth at 32°C for 24 h, patches were replica plated to nonselective agar plates; replica platings were repeated three times at 24-h intervals, and then patches were replica plated to selective agar plates. Solid growth of cells in a patch on nonselective plates indicates the plasmid genetic marker is as stably maintained as a chromosomal marker. Therefore, these clones potentially represent recombinants in which plasmid sequences have recombined with the yeast genome.

RESULTS

Several observations lead to the hypothesis that a recombination hot spot occurs in sequences adjacent to yeast *CEN14*. First, the genetic marker, *TRP1*, on plasmid pYe(SH)10 often segregates through meiosis and mitosis as a chromosomal marker (Neitz, Ph.D. thesis). Yeast sequences present on this plasmid in addition to the *TRP1* gene include the replicator *ARS1* and a 1.5-kb *Sal*I-*Hin*dIII fragment (SH) that originates from the centromere region of chromosome XIV (Fig. 1). The vector plasmid YRp7, which differs from pYe(SH)10 in that it lacks the SH DNA (Fig. 1), segregates through cell division as a typical autonomously replicating *ARS* plasmid (14). Second, Southern analysis of various yeast transformants in which the pYe(SH)10 plasmid genetic marker (*TRP1*) is stably maintained through cell division reveals that plasmid sequences are integrated in some cases or absent in others. These results could be explained if sequences contained within the SH fragment stimulate plasmid pYe(SH)10 to recombine with genomic sequences, thus allowing the *TRP1* gene to become a chromosomal marker. Finally, *ARS* plasmids containing SH DNA and a yeast centromere are unstable in yeasts and are structurally rearranged presumably owing to SH-stimulated recombination leading to the formation of dicentric plasmids.

Identification of CEN-linked hot spot in *S. cerevisiae*. In a standard patch test (see Materials and Methods), scoring *TRP1*$^+$ transformants obtained from either YRp7 or pYe(SH)10 DNAs, a relatively high number of pYe(SH)10 transformants displayed a stable Trp$^+$ phenotype (Table 1) (Neitz, Ph.D. thesis). To investigate the hypothesis that this phenomenon was due to frequent recombination between plasmid and genomic sequences, we examined yeast strains transformed with plasmid pYe(SH)10 by hybridization analysis. Putative recombinants were initially identified by a low level or total lack of detectable sequences homologous to nick-translated pBR322 DNA in a colony hybridization assay. Of 157 colonies assayed, 14 yielded signals that were indistinguishable from that produced by the untransformed yeast control. These 14 transformants were subjected to Southern analysis to characterize the nature of the genetic exchange. Total genomic DNA was cleaved with *Pvu*II and fractionated by gel electrophoresis. Since the only site in plasmid pYe(SH)10 recognized by *Pvu*II is in the pBR322 sequences, if plasmid DNA is integrated, two genomic *Pvu*II bands homologous to pBR322 are expected. However, if the plasmid is autonomous, a single band homologous to pBR322 and identical in size to linear plasmid is expected. The results of this analysis (Fig. 2) indicated that plasmid pYe(SH)10 is involved in two or more types of exchange event. Integrated plasmid was observed in several colonies (for example, Fig. 2A, lanes 2, 3, and 5). Plasmid was not detected in several *TRP*$^+$ colonies such as those represented in Fig. 2A, lanes 4 and 7, and Fig. 2B, lanes 1, 2, and 21, suggesting that plasmid was lost from the population after the occurrence of gene conversion or plasmid integration followed by excision. Several examples of colonies containing plasmid in both integrated and autonomous forms can be seen (e.g., Fig. 2A, lane 2, and Fig. 2B, lanes 8 and 9).

TABLE 1. Effect of relative orientation of SH and *TRP1 ARS1* DNA segments on hot spot activity

Plasmid[a]	No. of patches[b]	Stable Trp$^+$	% Stable Trp$^+$[c]
pYe(SH)10	172	13	8
pYe(SH)10′	126	0	0
pYe(ESH)10	65	0	0
pYe(ESH)10′	63	0	0
YRp7	75	1	1

[a] See Fig. 5 for plasmid structures and Materials and Methods for a description of the constructions.

[b] The number of independent plasmid transformants in yeast strain J17 subjected to the patch assay as described in Materials and Methods.

[c] The percentage of patches assayed in which the plasmid genetic marker (*TRP1*) is stably maintained. This number is a relative measure of the frequency with which plasmids recombine with yeast genomic sequences. The probability that the difference between the percentage observed for plasmid pYe(SH)10 and that of any of the other plasmids is due to sampling error is ≤5.2%.

In summary, results of hybridization analyses demonstrate that plasmid pYe(SH)10 frequently recombines with yeast genomic sequences and (i) integrates; (ii) integrates and then excises; or (iii) is lost from the population after a gene conversion event. In addition, any one colony may contain cells representative of more than one event. Since the control, vector plasmid YRp7, does not exhibit the unusual properties observed for plasmid pYe(SH)10 (Neitz, Ph.D. thesis), these results suggest that the 1.5-kb *Sal*I-*Hin*dIII chromosome XIV fragment acts to stimulate genetic exchange.

Dicentric assay for recombination. To test the hypothesis that the SH DNA mediates recombination, we used a dicentric assay. This assay is based on the fact that a single crossover between two circular *CEN* plasmids or between a *CEN* plasmid and a chromosome results in formation of dicentric structures, which undergo structural rearrangements in *S. cerevisiae* (9, 20, 32). Plasmid pMN105 (Fig. 3), which contains *CEN3* and the SH fragment, and plasmid pCH10d (see Materials and Methods), which contains *CEN14* and the SH fragment, were used in this analysis. If SH DNA stimulates homologous genetic exchange, these plasmids are expected to be rearranged in *S. cerevisiae* owing to formation of dicentric structures. However, if SH DNA does not promote crossing over, only intact plasmids should be recovered from yeast transformants. Plasmids were introduced by transformation into separate cultures of yeast strain J17. After several generations of growth selecting for the plasmid genetic marker, plasmids were recovered from individual yeast transformants and introduced into *E. coli* JA300. Plasmid was reisolated from *E. coli* and characterized by restriction map analysis. As a control, it was demonstrated that plasmids pMN105 and pCH10d were not rearranged in *E. coli* JA300. Plasmid was introduced directly into *E. coli* by transformation, isolated, and characterized. Plasmids from 12 individual JA300 transformants containing

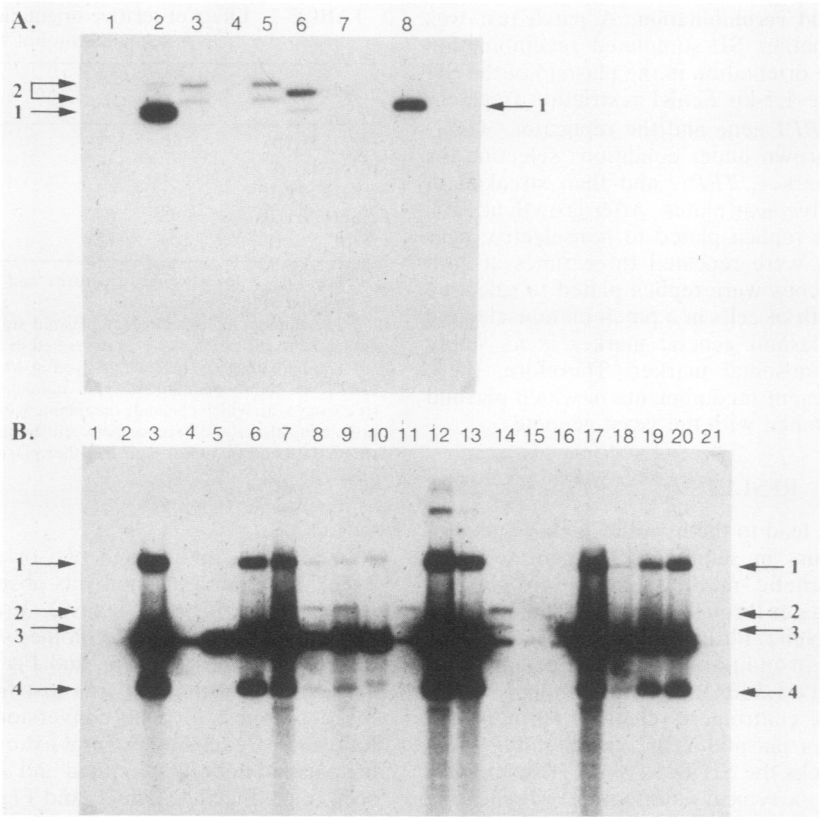

FIG. 2. Southern blot analysis of total genomic DNA isolated from yeast strains transformed with plasmid pYe(SH)10. DNAs in all lanes were cut with *Pvu*II. The probe was pBR322 [^{32}P]DNA. (A) DNAs were as follows: lane 1, an untransformed yeast strain; lanes 2 to 5 and 7, various pYe(SH)10 transformants; lane 6, a pYe(SR)10 transformant; lane 8, control plasmid pYe(SH)10. Arrows 1 and 2 indicate linear and integrated plasmid pYe(SH)10, respectively. Plasmid transformants were in yeast strain SB7883-1C. (B) DNAs were as follows: lanes 1 to 9 and 11 to 21, various pYe(SH)10 transformants in yeast strain J17; lane 10, control plasmid pYe(SH)10. Arrows 1 and 4 indicate nicked and supercoiled plasmid, respectively. Arrows 2 and 3 indicate integrated plasmid bands.

plasmid pMN105 and 12 transformants containing plasmid pCH10d were examined, and rearrangements were never observed to occur in this *E. coli* strain.

The results of the dicentric assay are shown in Table 2.

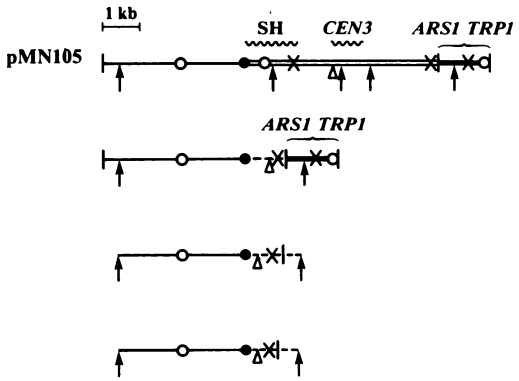

FIG. 3. Restriction maps of intact plasmid pMN105 and rearranged plasmids derived from pMN105. Restriction site symbols are as follows: +, *Eco*RI; X, *Hin*dIII; △, *Bam*HI; ●, *Sal*I; ○, *Acc*I; ↑, *Hin*cII. Thin lines represent pBR322 sequences, black bars and open bars represent the indicated yeast sequences, and the dashed lines represent DNA of unknown origin. Shown here is the rearranged form of three plasmids; however, 39 of the 42 pMN105 plasmids indicated in Table 2 were found to be similarly rearranged.

Plasmid DNAs recovered from 15 independent yeast transformants of plasmid pMN105 were used to transform *E. coli* JA300, and plasmids reisolated from 42 individual JA300 transformants (two to three *E. coli* transformants representing each yeast transformant) were characterized by restriction map analysis. Of 42 plasmids characterized, 39 were rearranged, and in the cases in which intact plasmid pMN105

TABLE 2. Structural instability of various *CEN* plasmids containing the SH recombinational hot spot

Plasmid[a]	No. of yeast transformants investigated[b]	No. of *E. coli* investigated[c]	% Rearranged[d]
pMN104	3	20	100
pCH10d	5	18	72
pMN105	15	42	93
pYe(RH)10	5	18	15
pYe(SH)10	5	21	0

[a] See Materials and Methods for plasmid constructions.

[b] Plasmids were isolated from the number of independent yeast transformants indicated. All transformants were in yeast strain J17.

[c] Plasmids were recovered by using total DNA isolated from yeast transformants to transform *E. coli* JA300. Indicated in this column are the total numbers of independent *E. coli* clones from which plasmids were isolated and characterized by restriction map analysis. Approximately equal numbers of *E. coli* clones were investigated for each of the independent yeast transformants.

[d] Percentage of the total number of plasmids mapped that were found to be rearranged.

was recovered, rearranged plasmid was also isolated from the same yeast transformants. The rearranged plasmids contained most or all of the pBR322 sequences present in the original plasmid (Fig. 3); however, the restriction map of the remaining DNA appears to be unrelated to that of intact pMN105. A similar result was obtained with plasmid pCH10d (Table 2). Plasmid pYE(SH)10, *CEN* plasmid pYe(RH)10, and the dicentric plasmid pMN104 which contains *CEN3* and *CEN14* (see Materials and Methods) were included in this assay as controls. Since plasmid pYe(SH)10 lacks a centromere, it should be recovered intact from yeasts regardless of whether the SH DNA stimulates homologous recombination. As expected, this plasmid was never observed to be rearranged in yeasts (Table 2). Dicentric plasmid pMN104 was always rearranged in yeasts, and *CEN* plasmid pYe(RH)10 (lacking SH sequences) was relatively rarely rearranged in yeasts (Table 2). These results support the hypothesis that SH DNA acts to increase the frequency of mitotic crossing over.

Genomic location of recombination event. Genetic exchange between plasmid pYe(SH)10 and the yeast genome could occur by homology to either or both the *TRP1 ARS1*

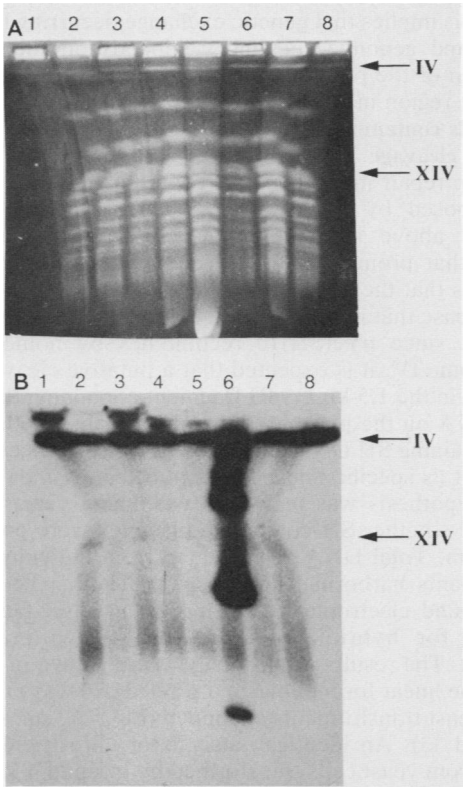

FIG. 4. OFAGE analysis to determine the chromosomal location of recombination between yeast genomic sequences and plasmid pYe(SH)10. (A) The OFAGE gel (1.2% agarose) was run at 300 V for 24 h with a 94-s pulse interval and stained with ethidium bromide. DNAs were as follows: lanes 1 to 3 and 8, various pYe(SH)10 transformants in yeast strain SB7883-1C; lane 4, untransformed yeast strain J17; lane 5, untransformed yeast strain SB7883-1C; lanes 6 and 7, pYe(SH)10 transformants in yeast strain J17. (B) Autoradiogram of gel shown in panel A after hybridization to ^{32}P-labeled *TRP1 ARS1* sequences. Arrows indicate chromosomes found at these positions in the gel. The position of chromosome XIV under the conditions used above was determined by probing gels with a chromosome XIV-specific probe. The picture (A) is magnified 1.1 times relative to the autoradiogram (B).

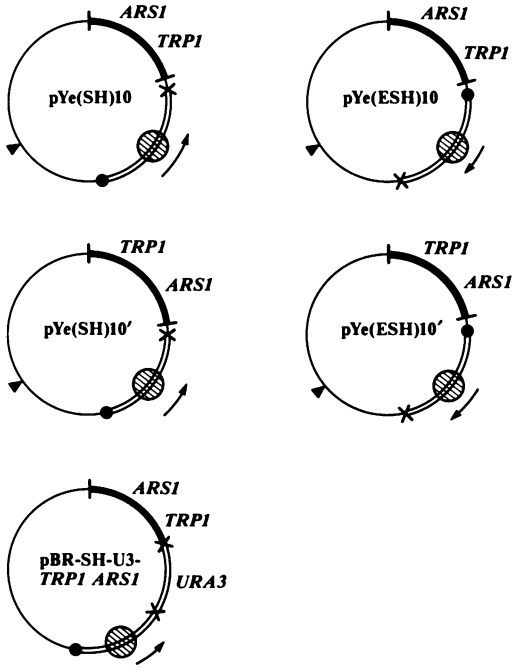

FIG. 5. Plasmids representing the four possible relative orientations of the *TRP1 ARS1* region and SH DNA. The restriction site symbols are: +, *Eco*RI; X, *Hin*dIII; ●, *Sal*I; ▼, *Pvu*II. Thick lines represent *TRP1 ARS1* sequences, thin lines represent pBR322 DNA, and the open bar represents SH DNA. The arrows designate the direction of migration of the putative recombination endonuclease.

sequence on chromosome IV (23) or the SH DNA on chromosome XIV. To identify the genomic location of the recombination event, we performed OFAGE on chromosomes isolated from recombinant yeasts. OFAGE gels were prepared for hybridization and probed with a 1.5-kb *Eco*RI restriction fragment containing yeast *TRP1 ARS1* sequences. If plasmid integrated by homology to the *TRP1 ARS1* region, only chromosome IV is expected to hybridize to the probe. However, if plasmid stimulates an exchange with chromosome XIV, the probe is expected to hybridize to both chromosomes IV and XIV. In seven of eight recombinant yeasts tested, only chromosome IV was observed to hybridize to the probe (results shown here are for six of eight recombinants assayed; Fig. 4, lanes 1 to 3 and 6 to 8). *TRP1 ARS1* sequences are present on at least three chromosomes in one yeast strain in which plasmid sequences could not be detected by Southern analysis (Fig. 4, lane 6). However, chromosome XIV was never observed to cross-hybridize to the *TRP1 ARS1*-containing DNA fragment, indicating that the SH fragment contains sequences that in some way stimulate recombination primarily within the *TRP1 ARS1* region.

SH DNA can stimulate homologous genetic exchange in plasmid yeast sequences other than the *TRP1 ARS1* region. To address the question of whether SH DNA stimulates recombination in yeast sequences other than the *TRP1 ARS1* region, we did two experiments. First, we determined whether an SH plasmid containing both the yeast *URA3* and *TRP1 ARS1* sequences integrates into the yeast genome. Second, we investigated the chromosomal location of integrated plasmids to determine whether integration had occurred at the *TRP1* locus (chromosome IV) or at the *URA3* locus (chromosome V). Plasmid pBR-SH-U3-*TRP1 ARS1* is

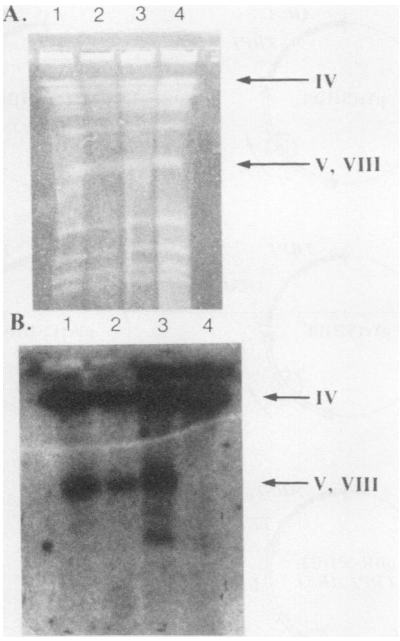

FIG. 6. OFAGE analysis to determine the chromosomal location of recombination between yeast genomic sequences and plasmid pBR-SH-U3-*TRP1 ARS1*. (A) The OFAGE gel (1.2% agarose) was run at 300 V for 24 h with a 50-s pulse interval and stained with ethidium bromide. DNAs were as follows: lanes 1 to 3, chromosomes isolated from different yeast strain J17 transformants harboring integrated plasmid pBR-SH-U3-*TRP1 ARS1*; lane 4, untransformed yeast strain J17. (B) Autoradiogram of gel shown in panel A after hybridization to ^{32}P-labeled *TRP1 ARS1* DNA. The uppermost band in lanes 3 and 4 is DNA remaining in the wells. The fastest-migrating band in lane 3 occurs at a position where no chromosome is found and is assumed to represent supercoiled plasmid pBR-SH-U3-*TRP1 ARS1*. Arrows indicate chromosomes found at these positions in the gel. The position of the chromosome V-VIII doublet under the conditions used above was confirmed by using probes specific for these chromosomes.

identical to plasmid pYe(SH)10 except that a 1.1-kb *Hin*dIII fragment containing the yeast *URA3* gene is inserted in the *Hin*dIII site between the *TRP1 ARS1* region and the SH DNA (Fig. 5). Yeast strain J17 was transformed with pBR-SH-U3-*TRP1 ARS1* DNA under conditions selecting for Ura$^+$ transformants. Recombinants were identified by subjecting single colonies to a patch test (see Materials and Methods). Of 203 colonies assayed, 19 were phenotypically stable Trp$^+$. These 19 colonies exhibited a stable Ura$^+$ phenotype as well, suggesting that the plasmid had integrated. Hybridization analysis performed on 11 of the stable Ura$^+$ Trp$^+$ transformants indicated that plasmid pBR-SH-U3-*TRP1 ARS1* is integrated in the genome of all 11 strains (Neitz, Ph.D thesis).

Chromosomes were isolated from three yeast transformants carrying integrated plasmid pBR-SH-U3-*TRP1 ARS1* and subjected to OFAGE analysis to determine the genomic location of integrated plasmid. The OFAGE gel was prepared for hybridization and probed with ^{32}P-labeled *TRP1 ARS1* sequences. This probe is expected to hybridize to chromosome IV regardless of whether integrated plasmid is present on this chromosome; however, if plasmid integrated by homology to the *URA3* region, chromosome V would also be expected to hybridize (23). The chromosome V-VIII doublet hybridizes to the labeled *TRP1* probe (Fig. 6, lanes 1 to 3), indicating that the plasmid is integrated in one or both of these chromosomes. Since pBR-SH-U3-*TRP1 ARS1* does not contain sequences derived from yeast chromosome VIII, it is most likely that this plasmid is integrated on chromosome V at the *ura3* locus. To determine whether the plasmid is integrated on chromosome IV as well, an OFAGE gel identical to that shown in Fig. 6 was prepared and probed with ^{32}P-labeled pBR322 DNA. The results of this experiment (not shown) revealed that plasmid pBR-SH-U3-*TRP1 ARS1* did not integrate on chromosome IV in the yeast strains tested. These findings suggest that homologous genetic exchange stimulated by SH DNA is not restricted to the *TRP1 ARS1* sequences, but can occur in other yeast sequences that are positioned adjacent to the SH fragment.

Orientation effects. A patch test was done to determine whether a specific relative orientation of the *TRP1 ARS1* fragment and the SH DNA is required for SH-stimulated recombination. Plasmids representing the four possible relative orientations of the *TRP1 ARS1*-containing *Eco*RI fragment and the SH DNA (Fig. 5) (Materials and Methods) were subjected to the patch test. The data (Table 1) indicate that only plasmid pYe(SH)10 is involved in a high-frequency recombination event. In this assay, plasmids pYe(SH)10′, pYe(ESH)10, and pYe(ESH)10′ did not appear to recombine at an elevated frequency compared with the control plasmid, YRp7. This implies that genetic exchange occurring between plasmid and genomic sequences depends on the relative orientation of the potential recombination site with respect to the SH region in the plasmid.

Plasmids containing SH fragment are linearized in *S. cerevisiae* by cleavage at specific sites. Based on the double-strand-gap repair mechanism of recombination in *S. cerevisiae* proposed by Szostak et al. (33) and on evidence presented above indicating that SH DNA contains sequences that promote mitotic recombination, a likely hypothesis is that the SH fragment acts as an entry site for an endonuclease that introduces a double-strand cleavage. Furthermore, since pYe(SH)10 recombines by homology to chromosome IV, it is expected that a putative cleavage site should be in the 1.5-kb *Eco*RI fragment containing the *TRP1 ARS1* DNA on the plasmid. According to this model, plasmids containing SH DNA and *TRP1 ARS1* sequences should be present as specific linear forms in *S. cerevisiae*.

This hypothesis was tested by examining yeast cells to determine whether SH-containing plasmids were present in linear form. Total DNA was isolated from individual yeast transformants harboring plasmid pYe(SH)10, pYe(SH)10′, or YRp7 and electrophoresed through agarose. Gels were prepared for hybridization and probed with pBR322 [^{32}P]DNA. The results of this analysis are shown in Fig. 7A and B. The linear form of plasmid pYe(SH)10 was present in several yeast transformants examined (Fig. 7A, lanes 3, 5, 8, 9, 11, and 13). An identical analysis of unrestricted DNA isolated from yeast cells transformed by plasmid YRp7 (Fig. 7B, lanes 1 to 4) and plasmid pYe(SH)10′ (data not shown) (Neitz, Ph.D. thesis) revealed that these plasmids also are present in linear form. Thus, all three plasmids examined are present as linear as well as circular molecules in *S. cerevisiae*.

A prediction of the model described above is that plasmid pYe(SH)10 should be linearized by cleavage at specific sites within the *TRP1 ARS1* region of the plasmid, whereas YRp7 should not be cut in this region. One possibility is that plasmid YRp7 is cut at random sites. To address this question, DNA was isolated from individual yeast transformants, restricted with *Pvu*II, and subjected to hybridization analysis. If the site of the in vivo double-strand cleavage is

specific, when gels are probed with labeled pBR322 DNA, discrete bands smaller in size than linear plasmid are expected. However, if cut sites are randomly located, a smear with greater mobility than linear plasmid is expected. The results of the hybridization analysis are shown in Fig. 7B. Genomic DNA isolated from *S. cerevisiae* transformed with plasmid pYe(SH)10 was cleaved with *Pvu*II and fractionated by gel electrophoresis. Four bands (indicated by the arrows 6 to 10) lower in molecular weight than linear plasmid (Fig. 7B, lane 18) are present in all three yeast transformants. A similar result was obtained with DNA isolated from yeast cells transformed with plasmid pYe(SH)10′ (Fig. 7B, lanes 12 to 14). The presence of several discrete bands with greater mobility than linear plasmid suggests that at least two specific cleavage sites occur in each of these plasmids. However, when *Pvu*II-cleaved genomic DNAs from cells harboring control plasmid YRp7 were subjected to a similar analysis, only linear plasmid was observed to hybridize to nick-translated pBR322 DNA (Fig. 7B, lanes 6 to 10). The absence of discrete bands below the linear YRp7 plasmid band indicates that sites recognized by yeast nucleases are randomly located in YRp7 sequences. Thus, it is concluded that the presence of SH DNA on a plasmid stimulates recognition and cleavage at specific sites in the plasmid by yeast endonucleases.

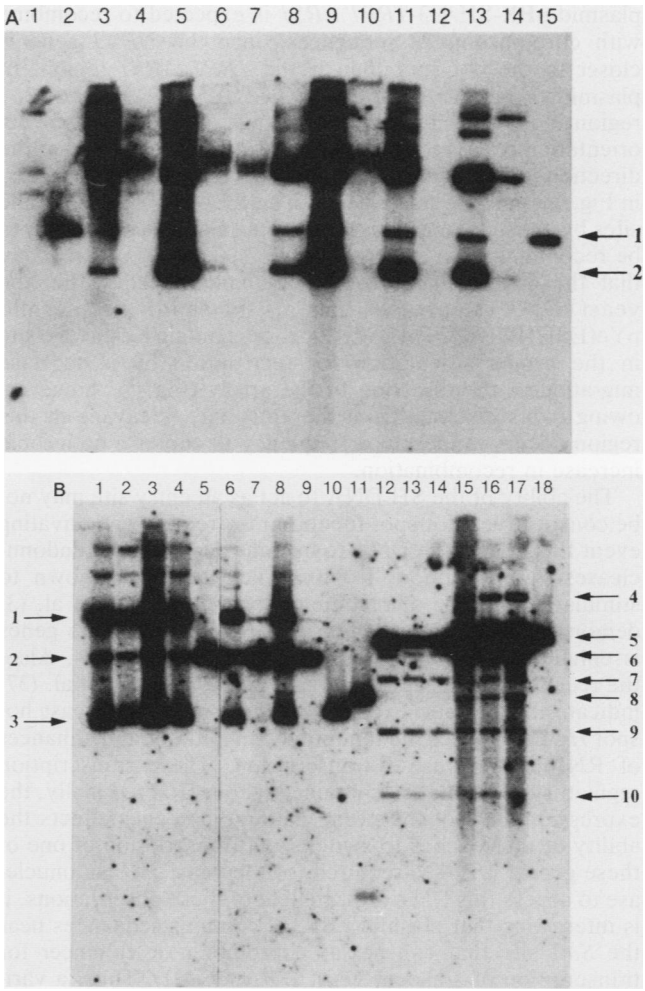

FIG. 7. Hybridization analysis of total DNA isolated from yeast cells harboring various plasmids. pBR322 [^{32}P]DNA was used as the probe. (A) Lanes: 1 and 14, unrestricted total genomic DNA isolated from yeast transformants containing plasmid pYe(SH)10′; 2 and 15, plasmid pYe(SH)10 DNA linearized with *Pvu*II; 3 to 13, unrestricted total genomic DNA isolated from various yeast transformants containing plasmid pYe(SH)10. Arrows 1 and 2 indicate linear and supercoiled plasmid, respectively. (B) Lanes: 1 to 4, uncut DNA isolated from four different yeast transformants harboring plasmid YRp7; 5, purified plasmid control YRp7 linearized with *Pvu*II; 6 to 9, same as lanes 1 to 4 except that DNAs were digested with *Pvu*II; 10 and 11, 4.0- and 4.4-kb size standards; 12 to 14, *Pvu*II-digested total genomic DNAs isolated from three different transformants harboring plasmid pYe(SH)10′; 15 to 17, *Pvu*II-digested total genomic DNAs isolated from three different yeast transformants harboring plasmid pYe(SH)10; 18, plasmid pYe(SH)10 DNA linearized with *Pvu*II. Plasmid transformants were in yeast strain J17. Arrows 1 to 3: nicked, linear and supercoiled plasmid YRp7 DNAs. Arrows 4 and 5: nicked and linear plasmid pYe(SH)10 or pYe(SH)10′ DNAs. Arrows 6 to 10: bands generated by cleavage of pYe(SH)10 and pYe(SH)10′ DNAs by yeast endonucleases.

DISCUSSION

We reported here the identification and characterization of a DNA fragment that contains sequences which promote mitotic recombination in the yeast *S. cerevisiae*. Briefly summarized, the evidence that hot spot activity is present on a 1.5-kb *Sal*I-*Hin*dIII fragment from the centromere region of chromosome XIV includes the following. (i) Plasmids containing this DNA and *CEN3* or *CEN14* are rearranged in yeast cells, presumably owing to the formation of dicentric structures; (ii) mitotic and meiotic analyses suggest that integration or gene conversion occur at an unusually high frequency in cells transformed with plasmid pYe(SH)10 or pBR-SH-U3-*TRP1 ARS1* compared with that in yeast cells transformed with control plasmid YRp7; (iii) Southern analysis of transformants of plasmid pYe(SH)10 revealed that this plasmid is involved in a variety of recombination events in yeast cells resulting in stable maintenance of the plasmid genetic marker *TRP1*; (iv) the presence of SH DNA on a plasmid results in cleavage at specific sites in the plasmid by yeast endonucleases.

Based on data presented in this paper, the following model is proposed. The SH DNA contains an entry site for a recombination endonuclease which recognizes specific DNA sequences hereafter referred to as cleavage sites. The nuclease migrates unidirectionally along the DNA in the direction of the arrow in Fig. 5. A characteristic of the cleavage site is that it is asymmetrical; thus, the set of sites recognized by an enzyme migrating clockwise is different from the set recognized when the enzyme is moving counterclockwise. When a cleavage site is recognized, the nuclease introduces a double-strand cut into the molecule. Cleavage sites occur in the *TRP1 ARS1*, *URA3*, and pBR322 sequences. Sometimes cleavage sites are encountered but not recognized and the nuclease continues to the next site; thus, in individual colonies, there is a population of linear plasmids cut at different locations. Only plasmids linearized by cleavage within yeast sequences are recombinagenic in *S. cerevisiae*. The probability of cutting at a particular cleavage site decreases with its distance from the entry site. According to this model plasmid pYe(SH)10 is expected to be highly recombinagenic in *S. cerevisiae* since the first cleavage site encountered by the recombination enzyme and therefore the site with the highest probability of being cut is in the *TRP1 ARS1* region (Fig. 5). Linearized plasmid pYe(SH)10 could integrate on chromosome IV or be involved in gene conversion by double-strand-gap repair. By the same argument,

plasmid pBR-SH-U3-*TRP1 ARS1* is expected to recombine with chromosome V sequences since the *URA3* gene is closer to the hot spot than is the *TRP1 ARS1* region. In plasmids pYe(SH)10′ and pYe(ESH)10, the *TRP1 ARS1* region does not contain a cleavage site in the correct orientation to be recognized by an enzyme migrating in the direction indicated in Fig. 5. Although the results presented in Fig. 7B indicate that plasmid pYe(SH)10′ is cut at specific sites by yeast endonucleases, this plasmid does not appear to be recombinagenic in yeast (Table 2). Thus, it seems likely that the double-strand breaks are not located within the yeast DNA sequences in this plasmid. In plasmid pYe(ESH)10′, *TRP1 ARS1* sequences contain a cleavage site in the proper orientation for recognition by a nuclease migrating in the direction of the arrow (Fig. 5); however, owing to its distance from the entry site, cleavage in this region occurs at too low a frequency to confer a noticeable increase in recombination.

The ability of the SH DNA to act as an entry site may not be constitutive. Hot spot function may require an activating event that allows the DNA to become accessible to endonucleases. Transcription, for example, has been shown to stimulate a variety of recombinations. Blackwell et al. (3) demonstrated that rearrangement of immunoglobulin genes is enhanced by transcription in adjacent sequences. Also, the recently published results of Voelkel-Meiman et al. (37) indicate that genetic exchange stimulated by the yeast hot spot *HOT1* requires the function of an initiator and enhancer of RNA polymerase I transcription. These transcription regulatory elements are contained within *HOT1*. Finally, the expression state of the yeast mating-type genes affects the ability of these genes to switch, and transcription of one of these genes, *MAT*, is required for the yeast *HO* endonuclease to access this DNA (16). In light of these observations, it is interesting that plasmid pBR322 contains sequences near the *Sal*I site that can act as a promoter or enhancer for transcription of adjacent yeast DNA (26, 31). Thus, a variation of the model presented above is that SH-stimulated recombination is due to transcription into SH DNA that is promoted or enhanced by pBR322 sequences.

One conspicuous feature of the hot spot contained within the SH DNA fragment is that it originates from a region of the yeast genome immediately adjacent to a centromere (Fig. 1A) (24). The data presented here suggest that sequences within the SH DNA act in vivo to stimulate mitotic recombination in the *CEN* region of chromosome XIV. Thus, it is interesting to note the report of a diploid yeast strain trisomic for one arm of chromosome XIV (15). While one could envision several tangible mechanisms by which this trisomy could have arisen, one possibility is that it is a result of an SH-stimulated double-strand cleavage occurring near *CEN14*. Healing of the cut end by the addition of telomeres would allow the arm that retained the centromere to be maintained by the cell.

In several organisms including *Aspergillus nidulans* (27), *Neurospora stinophila* (25), *Schizosaccharomyces pombe* (21), and *S. cerevisiae* (17, 19), the relative frequency of mitotic recombination is greater in some *CEN*-linked intervals than is meiotic exchange in these regions. Thus, for these intervals, the meiotic and mitotic map distances do not agree. It was recently observed that in *S. cerevisiae* the ability of the centromere to repress meiotic recombination in adjacent DNA can account, at least in part, for the discrepancies in map distances (17). Evidence reported in this paper suggests that genetic elements such as that found adjacent to yeast *CEN14* may also contribute to these differences.

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