MOLECULAR AND CELLULAR BIOLOGY, June 1993, p. 3125–3134
0270-7306/93/063125-10$02.00/0

Vol. 13, No. 6

A 5′-3′ Exonuclease from *Saccharomyces cerevisiae* Is Required for In Vitro Recombination between Linear DNA Molecules with Overlapping Homology

KIMBERLY N. HUANG AND LORRAINE S. SYMINGTON*

Institute of Cancer Research and Department of Microbiology, Columbia University College of Physicians and Surgeons, 701 West 168th Street, New York, New York 10032

Received 18 November 1992/Returned for modification 27 January 1993/Accepted 26 February 1993

When two linear DNA molecules with overlapping, homologous ends were incubated with a yeast nuclear extract, they recombined at the region of homology to produce a joint molecule. We have identified a 5′-3′ exonuclease in the extract that is likely to be responsible for the formation of the observed product. We propose that the exonuclease degrades each substrate to reveal regions of complementary sequence which anneal to form a recombinant product. Consistent with this model, we have partially purified the activity that promotes joint molecule formation and found it to cofractionate with a 5′-3′ exonuclease activity through three consecutive chromatography steps. We have further characterized the reaction to determine the optimal length of homology. Substrates with homologous terminal overlaps of 29 to 958 bp were capable of product formation, whereas substrates with longer overlaps were not. Extracts prepared from a number of recombination-defective or nuclease-deficient strains revealed no defect in exonuclease activity, indicating that the reaction is likely to be dependent upon the product of an as yet unidentified gene.

The importance of strand-specific exonucleases in genetic recombination has been well documented. In prokaryotes, the *recE* gene product (exonuclease VIII), the T7 gene 6 product, and λ exonuclease are all 5′-3′ double-stranded exonucleases proven to play a role in recombination (reviewed in reference 41). Several models for recombination in eukaryotes also predict the involvement of exonucleases. In the double-strand break repair (DSBR) model, it is proposed that the DNA ends created at the break are degraded by a strand-specific exonuclease to create single-stranded tails which are able to invade homologous DNA, thus initiating the recombination process (51). In vivo studies have documented 5′-3′ degradation of the broken ends formed at several double-strand breaks associated with recombination hot spots in the yeast *Saccharomyces cerevisiae*. After HO endonuclease cleavage at the *MAT* locus during mating-type switching, the DNA undergoes exonucleolytic degradation to produce a 3′ tail on the distal side of the HO cut (55). This strand is believed to invade the intact donor of mating-locus information, thus initiating transfer of information. A double-strand break induced at the *ARG4* locus during meiosis is processed to leave a 3′ single-stranded tail (48). This site has been shown to be a recombination initiation site (34), indicating that a 3′ tail may be involved in the initiation of meiotic recombination. Similarly, a meiotic recombination hot spot created by the insertion of the *LEU2* gene at the *HIS4* locus is associated with a meiosis-specific double-strand break that is processed to generate 3′ single-stranded tails (5, 7).

An exonuclease is also envisioned to act in the single-strand annealing (SSA) model of recombination, which was proposed to explain certain recombination events in mammalian systems (24–26). In this model, strand-specific degradation by an exonuclease is predicted to expose a complementary single strand on each of two linear, homologous DNA substrates, such that pairing can occur. As DNA is lost in the reaction, it is considered to be a nonconservative mechanism. Experiments with *Xenopus laevis* oocytes (8, 29–31) support a similar model in this system. In oocytes, the reaction has been characterized further to show that a 5′-3′ exonuclease degrades injected DNA, producing 3′ single-stranded tails which participate in recombination (29–31).

Several studies in *S. cerevisiae* are similarly consistent with the SSA model. When a double-strand break is introduced within one of two genes in direct repeat or between them, repair frequently occurs to produce a single copy of the gene, with loss of the intervening sequence. This has been shown for two copies of the *lacZ* gene in direct repeat on a plasmid (40), for duplicated chromosomal *ura3* genes (47), and at the repetitive ribosomal DNA (rDNA) and *CUP1* loci (35). In each case, it is proposed that strand-specific degradation occurs from the cut site until complementary strands of the two repeats can pair. Evidence of exonucleolytic processing has been demonstrated at the cut site both in the plasmid system (14) and at the chromosomal *ura3* duplication (47). The *RAD52* gene product is not required for the repair reaction at the rDNA locus (35). Even in the absence of an artificially generated double-strand break, recombination events that result in loss of an inserted marker from the rDNA locus are *RAD52* independent (10), suggesting that spontaneous recombination within natural repeats may occur by the SSA pathway. The requirement for Rad52 in this recombination pathway appears to depend on the number of direct repeats in the array, the length of the repeats, and the amount of DNA separating the repeats (14). Because Rad52 is required for events consistent with the DSBR model of recombination in *S. cerevisiae* (15, 51), it is likely that SSA is an alternative to the DSBR pathway and that the choice of pathway is dependent upon the substrates involved. This view is supported by the observation that the ratio of repair events occurring by these two pathways can be altered by varying the length of DNA separating the repeats (14). Repair between closer repeats proceeds primar-

* Corresponding author.

TABLE 1. Yeast strains used in this study

Strain	Genotype	Source or reference
W303a	*MAT***a** *ade2-1 leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1*	R. Rothstein
W303α	*MATα ade2-1 leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1*	R. Rothstein
LSY150	*MAT***a** *ade2-1 leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1 pep4::URA3*	This study
LSY151	*MATα ade2-1 leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1 pep4::URA3*	49
LSY267	*MAT***a** *ade2-1 leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1 pep4::LEU2*	This study
W838-24D	*MAT***a** *ade2-1 leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1 rad1::LEU2 rad52::TRP1*	J. McDonald and R. Rothstein
LSY140	*MATα ade2-1 leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1 rad1::LEU2 pep4::URA3*	This study
LSY141	*MAT***a** *ade2-1 leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1 rad52::TRP1 pep4::URA3*	This study
LSY142	*MAT***a** *ade2-1 leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1 rad1::LEU2 rad52::TRP1 pep4::URA3*	This study
LSY195	*MAT***a** *ade2-1 leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1 pep4::LEU2 rad50::URA3*	This study
LSY196	*MAT***a** *ade2-1 leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1 pep4::LEU2 rad50S::URA3*	This study
LSY197	*MAT***a** *ade2-1 leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1 pep4::LEU2 sep1::URA3*	This study
RS441	*MAT***a** *leu2-3 ura3-52 top1-1 trp1 nuc1::LEU2*	R. Sternglanz
LSY272-4A	*MATα ade2-1 leu2 his3-11,15 ura3 trp1 nuc1::LEU2*	This study
LSY346-5A	*MATα ade2-1 leu2 his3-11,15 ura3 trp1 nuc1::LEU2 sep1::URA3 pep4::LEU2*	This study

ily by SSA, whereas DSBR is favored when the repeats are separated by more than 6 kb.

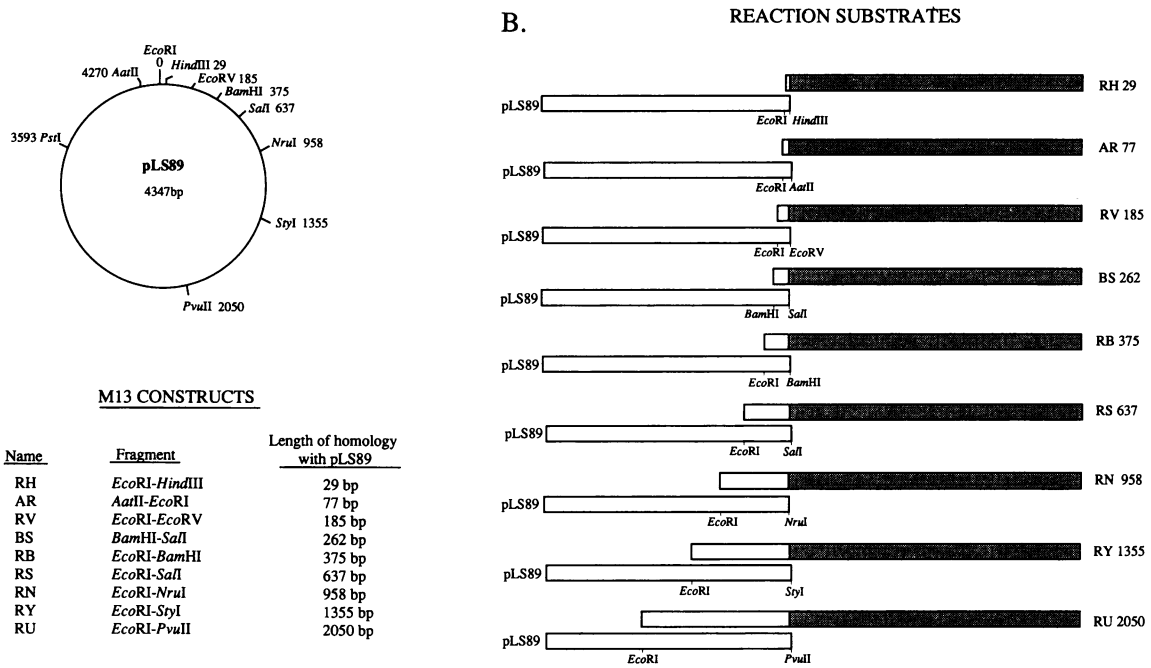
In vitro recombination and repair systems using yeast extracts have been described previously (49, 50). We have shown that nuclear extracts catalyze recombination between homologous linear and circular DNA substrates (49). In this study, we have used yeast nuclear extracts to stimulate recombination between two DNAs with overlapping, homologous ends. We show that DNA in this reaction is acted upon by a 5′-3′ strand-specific exonuclease, which is likely to be responsible for formation of the observed product.

MATERIALS AND METHODS

Strains and media. Media for *S. cerevisiae* were prepared as described by Sherman et al. (44). Yeast strains are listed in Table 1. New strain constructions are as follows. Strains LSY150 and LSY151 were made by transformation (17) of W303a and W303α, respectively, with plasmid pTS15 (38), using the one-step transplacement method (39). Strain LSY267 was made by transformation of W303a with plasmid pTS17 by using one-step transplacement. Strains LSY140, LSY141, and LSY142 were made by crossing LSY151 to the isogenic strain W838-24D and sporulating to create the haploid progeny containing *pep4* disruptions and the *rad* mutations indicated in Table 1. Strains LSY195 and LSY196 were made by one-step transplacement in LSY267, using plasmids pNKY83 and pNKY349 (1), respectively. Strain LSY197 was constructed by one-step transplacement of LSY267, using plasmid pRDK227. All strains created by transplacement were confirmed by Southern analysis. A strain containing a disruption of the *NUC1* gene (RS441) was backcrossed to W303 twice, and a spore carrying the *nuc1::LEU2* gene, LSY272-4A, was then crossed to LSY197. Haploid segregants obtained from this cross that were *sep1::URA3 nuc1::LEU2 pep4::LEU2* should be at least 87.5% isogenic with W303 and were used for fractionation of yeast extracts. *Escherichia coli* strains were grown at 37°C in LB medium supplemented with ampicillin (100 μg/ml). Plasmids and M13mp18 constructs were propagated in either *E. coli* XL1-Blue {*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*^{q}ZΔ*M15* Tn*10* (Tet^r)]} or *E. coli* JM109 [*recA1 endA1 gyrA96 thi-1 hsdR17* (r_K^- m_K^+) *supE44 relA1* Δ(*lac-proAB*) (F′ *traD36 proAB lacI*^{q}ZΔ*M15*)].

Construction of substrates. Plasmid pLS89 (Fig. 1A) was constructed by deleting the 14-bp *Ban*II fragment from pBR322 (49). All M13 constructs were made by cloning fragments of pLS89 into the polylinker of M13mp18. Construct RV contains the 185-bp *Eco*RI-*Eco*RV fragment of pLS89 cloned into *Eco*RI-*Hin*cII-cut M13mp18. Construct BS contains the 262-bp *Bam*HI-*Sal*I fragment of pLS89 cloned into *Bam*HI-*Sal*I-cut M13mp18. Construct RB contains the 375-bp *Eco*RI-*Bam*HI fragment of pLS89 cloned into *Eco*RI-*Bam*HI-cut M13mp18. Construct RN contains the 958-bp *Eco*RI-*Nru*I fragment of pLS89 cloned into *Eco*RI-*Hin*cII-cut M13mp18. Construct RU contains the 2,050-bp *Eco*RI-*Pvu*II fragment of pLS89 cloned into *Eco*RI-*Hin*cII-cut M13mp18. Construct RH was made by digesting construct RB with *Hin*dIII and religating. Construct AR was made by first cloning the 3,389-bp *Nru*I-*Eco*RI fragment of pLS89 into *Eco*RI-*Hin*cII-cut M13mp18. This construct was then digested with *Aat*II and *Pst*I, the ends were made blunt with T4 DNA polymerase, and the DNA was religated. Construct RS was made by first cloning the 3,593-bp *Eco*RI-*Pst*I fragment of pLS89 into M13mp18. This construct was then digested with *Pst*I and *Sal*I, the ends were made blunt with T4 DNA polymerase, and the DNA was religated. Construct RY was also made using the 3,593-bp *Eco*RI-*Pst*I fragment of pLS89 cloned in M13mp18, but digestion was with *Pst*I and *Sty*I prior to treatment with T4 DNA polymerase and religation. All substrate DNAs were prepared by the alkaline lysis protocol of Birnboim and Doly (4) and further purified by centrifugation in CsCl-ethidium bromide density gradients (42).

Preparation of yeast extracts. The method used for the preparation of yeast nuclear extracts is a modification of the procedure developed in this laboratory (49). Yeast cultures were grown in YEPD with shaking to a cell density of 5×10^7/ml. The cells were harvested by centrifugation at 3,000 × *g* for 5 min at 4°C in a Sorvall GSA rotor. The cells were washed with 100 ml of 1 M sorbitol per liter of original culture, weighed, and resuspended in 4 ml of S buffer (1.1 M sorbitol, 20 mM potassium phosphate [pH 7.0], 0.5 mM $CaCl_2$) per g of cells. Zymolyase 100T (Seigaku America Inc.) was added at 0.5 mg/ml, and protease inhibitors were added in the following final concentrations: phenylmethylsulfonyl fluoride (PMSF), 0.5 mM; pepstatin A, 1 mg/liter; and leupeptin, 0.5 mg/liter. These protease inhibitors were added to all solutions used in subsequent steps as well. Cells were incubated at 30°C with gentle agitation until >90%



M13 CONSTRUCTS

Name	Fragment	Length of homology with pLS89
RH	*Eco*RI-*Hind*III	29 bp
AR	*Aat*II-*Eco*RI	77 bp
RV	*Eco*RI-*Eco*RV	185 bp
BS	*Bam*HI-*Sal*I	262 bp
RB	*Eco*RI-*Bam*HI	375 bp
RS	*Eco*RI-*Sal*I	637 bp
RN	*Eco*RI-*Nru*I	958 bp
RY	*Eco*RI-*Sty*I	1355 bp
RU	*Eco*RI-*Pvu*II	2050 bp

FIG. 1. Plasmid and M13 constructs. (A) Plasmid pLS89; (B) reaction substrates. Nine fragments of pLS89 (white regions) were cloned into M13mp18 (shaded regions). Linearization of pLS89 and an M13 construct with the appropriate enzymes generates a substrate pair with terminal homology of variable length.

spheroplasts were obtained. The spheroplasts were collected by centrifugation at 1,000 × *g* for 5 min at 4°C, washed twice with 3 ml of SPC buffer [1 M sorbitol, 20 mM sodium piperazine-*N*,*N*′-bis(2-ethanesulfonate) (PIPES; pH 6.3), 0.1 mM $CaCl_2$] per g of original cell weight, and then resuspended in 0.25 ml of SPC per g. The resuspended spheroplasts were slowly added to 15 ml of ice-cold FL buffer (18% [wt/wt] Ficoll 400, 20 mM PIPES [pH 6.3], 0.5 mM $CaCl_2$) per g of original cell weight and stirred on ice for 20 min to allow lysis to occur. Cell debris was removed by two rounds of centrifugation at 3,000 × *g* in a Sorvall SS34 rotor for 10 min at 4°C. The nuclei were collected by centrifugation at 20,000 × *g* in a Sorvall SS34 rotor for 15 min at 4°C. Nuclei were resuspended in 15 ml of SPC per 500 ml of original culture, spun at 10,000 × *g* for 10 min in an SS34 rotor at 4°C, and then resuspended in 1 ml of L buffer [50 mM Tris-HCl (pH 7.5), 10 mM $MgSO_4$, 1 mM EDTA, 1 mM dithiothreitol (DTT), 2 mM spermidine, 0.5 M $(NH_4)_2SO_4$] per g of original cell weight. The nuclei were incubated on ice for 15 min with occasional gentle agitation. The lysate was centrifuged at 100,000 × *g* in a Beckman 50Ti rotor for 30 min at 4°C. Proteins were precipitated from the resulting supernatant by the slow addition of solid $(NH_4)_2SO_4$ to 55% saturation. The protein precipitate was collected by centrifugation at 20,000 × *g* for 10 min. The protein pellets obtained were either used immediately or stored for no more than 20 h at −80°C. The protein pellet was resuspended in buffer H [50 mM potassium *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonate (HEPES; pH 7.8), 1 mM EDTA, 1 mM DTT, 10% glycerol] prior to use in the recombination assays.

Whole cell extracts were prepared by lysis with glass beads as described by Wootner et al. (56), to the ammonium sulfate fractionation step. The procedures for preparation and fractionation of extracts were performed at 0 to 4°C unless otherwise stated. Protein pellets were resuspended at 50 mg/ml in buffer A [20 mM HEPES (pH 7.6), 1 mM EDTA, 10% (vol/vol) glycerol, 1 mM DTT, 0.5 mM PMSF, 1 μg of pepstatin A per ml, 0.5 μg of leupeptin per ml]. This extract was desalted either by extensive dialysis or by gel filtration chromatography on Sephadex G-25 to yield fraction II. For further fractionation of extracts, 150 mg of total protein from fraction II was applied to a 25-ml DEAE-cellulose (Whatman) column equilibrated with buffer A. Proteins were eluted with a 120-ml gradient of 0 to 0.3 M NaCl in buffer A. Active fractions were pooled (44 to 70 mM NaCl; 7.02 mg of total protein) and precipitated with solid ammonium sulfate added to 60% saturation (fraction III). Proteins were suspended in 0.6 ml of buffer A containing 0.15 M NaCl, and 0.5 ml of this fraction was applied to a 24-ml Superose 12 gel filtration column (Pharmacia LKB Biotechnology). Proteins were eluted in buffer A containing 0.15 M NaCl. A single broad peak of activity that corresponded with the major protein peak (fraction IV) was obtained. These fractions were pooled (1.68 mg of total protein) and applied to a 1.5-ml heparin agarose (Sigma) column equilibrated with buffer A containing 0.15 M NaCl. Proteins were eluted with a 20-ml gradient of 0.15 to 0.6 M NaCl in buffer A. A single peak of activity that catalyzed joint molecule formation eluted at 300 to 360 mM NaCl (fraction V).

Reaction conditions. Complete reaction mixtures (15 to 100 μl) contained 35 mM HEPES (pH 7.8), 10 mM $MgCl_2$, 5 mM ATP, 2 mM spermidine, 2 mM DTT, 0.5 mM NAD, 50 μM each CTP, UTP, and GTP, 20 μM deoxynucleoside triphosphates, 100 μg of bovine serum albumin per ml, and 40 μg of total substrate DNA per ml. The nuclear extract was added to a protein concentration of 0.75 to 1.0 mg/ml, and the reaction was carried out at 30°C for the specified time. Following the reaction, DNA was purified by extraction with phenol-chloroform-isoamyl alcohol (25:24:1), extraction with chloroform-isoamyl alcohol (24:1), and ethanol precipitation. After resuspension in Tris-EDTA, the equivalent of 250 to 320 ng of substrate DNA was used for Southern

analysis. Reactions using column fractions were terminated by the addition of 0.1 volume of stop solution (1 mg of proteinase K per ml, 2% [wt/vol] sodium dodecyl sulfate [SDS], 100 mM EDTA [pH 8.0]) and incubated for 20 min at 30°C. These samples were then used for Southern analysis without phenol extraction or ethanol precipitation. For dot blot analysis, pLS89 was linearized with *SalI* and incubated with column fractions for 40 min at 30°C. The reactions were terminated by the addition of 0.1 volume of stop solution as described above.

Southern analysis. Agarose gels were prepared and run in TAE (40 mM Tris acetate, 1 mM EDTA [pH 7.5]) containing 0.5 μg of ethidium bromide per ml. After electrophoresis, DNA was transferred to nylon membrane (Hybond N; Amersham) and hybridized with probes either made by random priming (13) or, in the case of oligonucleotide probes, labeled with [γ-^{32}P]ATP and polynucleotide kinase (37). Oligonucleotide sequences are as follows: oligo 1,5′-CGATCTTCCCCATCG-3′; and oligo 2,5′-CGATGGGGA AGATCG-3′. These sequences correspond to nucleotides 457 to 471 of both pBR322 and pLS89. Southern analysis was performed by standard methods (45). Gel transfer without denaturation was performed as described by Sun et al. (48) and Lichten and Fox (23). These gels were soaked in 1.5 M NaCl–0.5 M Tris-HCl (pH 7.2) for 1 h prior to transfer to membranes. Hybridization of blots probed with random-primed probes was performed at 65°C in a solution containing 6× SSCPE (1× SSCPE is 0.12 M NaCl, 15 mM sodium citrate, 13 mM KH_2PO_4, and 1 mM EDTA), 5× Denhardt's solution (1× Denhardt's solution is 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), and 0.5% SDS. For dot blot analysis, reaction mixtures containing pLS89 digested with *SalI* were adjusted to 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and applied to a nylon membrane in a dot blot manifold (Schleicher & Schuell). After being washed with 10× SSC, the membrane was baked at 80°C for 60 min and hybridized with either oligo 1 or oligo 2. Hybridization of blots probed with oligonucleotide probes was performed at 42°C in a solution containing 10% formamide, 6× SSCPE, 5× Denhardt's solution, and 0.5% SDS.

Exonuclease assays. *SalI*-linearized pLS89 was uniformly labeled in a random-priming reaction (12), and 200 pg (total of 160,000 cpm) of labeled DNA was added to each 20-μl reaction containing the complete reaction buffer. After 40 min, the reaction was terminated by the addition of 40 μl of stop solution (25 mM sodium pyrophosphate, 20 mM EDTA, 25 μg of calf thymus DNA per ml) and 50 μl of 20% trichloroacetic acid. After 10 min on ice, the precipitated DNA was removed by centrifugation and the radiolabel remaining in the supernatant was determined by scintillation counting.

RESULTS

Recombination is stimulated by yeast nuclear extracts. Reaction substrates were constructed by cloning fragments of plasmid pLS89 into M13mp18, a vector with no significant homology to pLS89. When pLS89 and an M13-based construct are made linear with the appropriate restriction endonucleases, a substrate pair which has overlapping, homologous ends is generated (Fig. 1B). Nine different M13 constructs with regions homologous to pLS89 ranging from 29 to 2,050 bp were generated. When linear substrates with overlapping homologous ends were incubated with yeast nuclear extract, they were found to recombine at the region of homology, producing a joint molecule. All nine substrate pairs were assayed for the ability to form product (Fig. 2) by Southern analysis using a 753-bp *PstI-EcoRI* fragment of pLS89 as the probe. This fragment hybridizes to pLS89 (4.4 kb) and the product DNA (11.6 kb) but not to the M13-based constructs (Fig. 2). An additional band of 8.7 kb which corresponds to the formation of an end-joined product described previously (49) was detected. In this experiment, it is apparent that substrates sharing homology ranging from 262 to 637 bp gave optimum product formation. To determine whether the two substrates were joined covalently, product DNA was analyzed under denaturing conditions. An intact product band was not detected, indicating that the junctions are not repaired by ligation (data not shown).

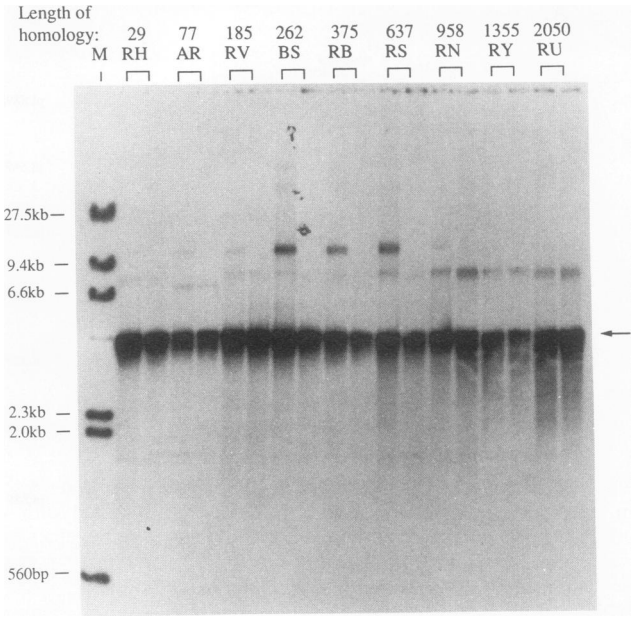


FIG. 2. Product formation. Each of the nine pairs of substrates was incubated with yeast nuclear extracts for 45 min. For each substrate pair, two lanes have been loaded. The left lane for each pair is the true reaction; the right lane is a control in which the two substrates were incubated separately in the extract and mixed just prior to loading. The probe is the 752-bp *EcoRI-PstI* fragment of pLS89, which recognizes the 4.4-kb band corresponding to the substrate pLS89 (lower arrow) and the 11.6-kb product band (upper arrow). The 8.7-kb band present in all lanes corresponds to the joining of two pLS89 molecules, an extract-dependent reaction described previously (49). The 7.2-kb band recognized in the AR reactions is the M13 substrate AR, which is the only M13 substrate which overlaps with the pLS89 fragment used as the probe. Lane M, size markers.

Several lines of evidence indicate that the reaction depends upon DNA sequence homology and is not the result of simple end-to-end ligation. First, when linear pLS89 was incubated with linear M13mp18 which contains no pLS89 insert, no product was formed (Fig. 3, lane 4). Second, analysis of the product by restriction endonuclease mapping using sites flanking the homologous region indicated that there was only one copy of this region (not shown). A direct end-to-end ligation would have resulted in a duplication of the homologous DNA. Third, if the reaction were a direct ligation, there would have been no bias in product formation for substrates with certain lengths of homology, as was observed.

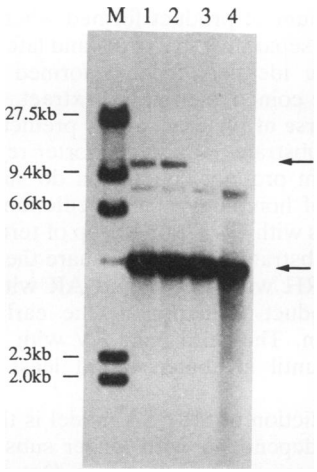


FIG. 3. Ability of substrates incubated separately in extract to form product. pLS89 linearized with *Sal*I and BS linearized with *Bam*HI were incubated in extract either separately or together. After phenol extraction, the substrates which had been incubated separately were mixed and coprecipitated. Lanes: M, size markers; 1, substrates incubated together in extract; 2, substrates incubated separately and later coprecipitated; 3, substrates incubated separately and mixed just prior to loading the gel; 4, pLS89 linearized with *Sal*I and linear M13mp18 incubated together in extract, which do not form product. The probe is the same as that used for Fig. 2. Arrows indicate the substrate pLS89 (lower arrow) and the product DNA (upper arrow).

Identification of a 5′-3′ exonuclease in the extract. Neutral gel transfer was performed to detect degradation of DNA by a strand-specific exonuclease (23, 48). Duplex DNA which has been separated on an agarose gel and blotted to nylon membrane without prior treatment in NaOH will fail to bind to the membrane. In contrast, DNA with a single-stranded tail will be capable of binding, and the polarity of the tail can be determined by hybridization with a complementary probe. Two complementary oligonucleotide probes were used to detect the presence and polarity of single-stranded DNA. pLS89 cut with *Sal*I was incubated with extract and then digested with *Pst*I to generate a 1.4-kb fragment containing the sequence complementary to the oligonucleotide probes. The substrate hybridized with oligo 1 but not with oligo 2, indicating the presence of a 3′ tail (Fig. 4). In this experiment, we also tested the M13 construct BS, which had been linearized with *Bam*HI to give an end with polarity opposite to pLS89 cut with *Sal*I. In this case, the 3′ tail hybridized to oligo 2 but not to oligo 1, confirming that this substrate is also the target of a 5′-3′ exonuclease.

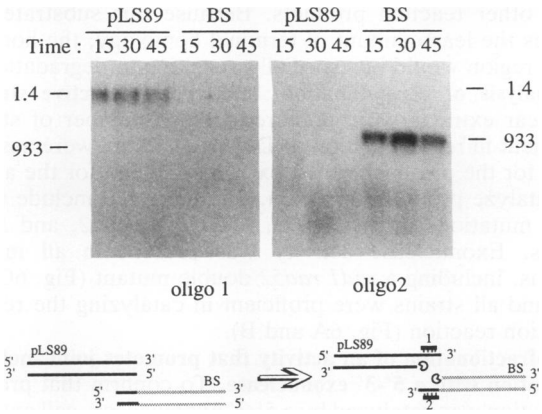


FIG. 4. Identification of 5′-3′ exonuclease activity. DNA which has been transferred to a nylon membrane without prior denaturation will be recognized by a probe only if the blotted DNA is single stranded. In the first three lanes of both blots, pLS89 linearized with *Sal*I was incubated with extract for the time indicated. After the reaction, the DNA was cleaved with *Pst*I to generate a 1.4-kb fragment recognized by the oligonucleotide sequence. In the next three lanes of both blots, the M13 construct BS was linearized with *Bam*HI, incubated with extract for the times indicated, and then cleaved with *Bgl*II to generate a 933-bp fragment recognized by the oligonucleotides. Complementary oligonucleotide probes were used on duplicate gels. Hybridization of oligo 1 to pLS89 and hybridization of oligo 2 to BS indicate the presence of 3′, single-stranded tails.

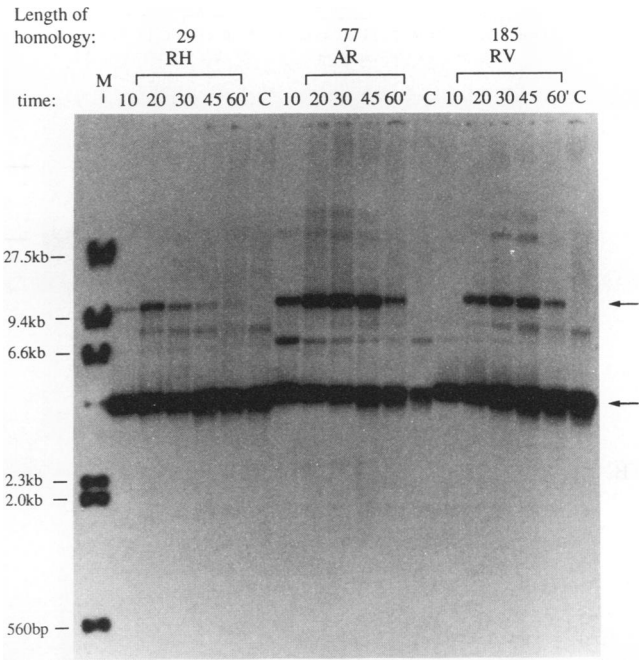


FIG. 5. Time course of the reaction. Substrates were pLS89 linearized with *Hin*dIII and RH linearized with *Eco*RI (29 RH), pLS89 linearized with *Aat*II and AR linearized with *Eco*RI (77 AR), and pLS89 linearized with *Eco*RV and RV linearized with *Eco*RI (185 RV). Homology shared between these substrate pairs is 29, 77, and 185 bp, respectively. Substrates were incubated with extract for the times indicated. Lanes C, control lanes in which the substrate DNAs were incubated separately in extract and mixed just prior to loading; lane M, size markers. Arrows indicate the substrate pLS89 (lower arrow) and the product DNA (upper arrow). The 7.2-kb band recognized in the AR reactions is the M13 substrate AR, which is the only M13 substrate which overlaps with the 752-bp *Eco*RI-*Pst*I pLS89 fragment used as the probe.

Substrates incubated separately with extract can later be mixed to form product. The SSA model predicts that degradation by an exonuclease exposes complementary strands which are capable of annealing. We reasoned that if this mechanism is responsible for product formation in our assay, separate incubation of the two substrates with extract should produce DNA molecules with single-stranded tails which can anneal to form product after removal from the extract. To test this prediction, the two substrates were incubated separately in extract, phenol extracted to remove proteins, mixed, and precipitated with ethanol. As shown in

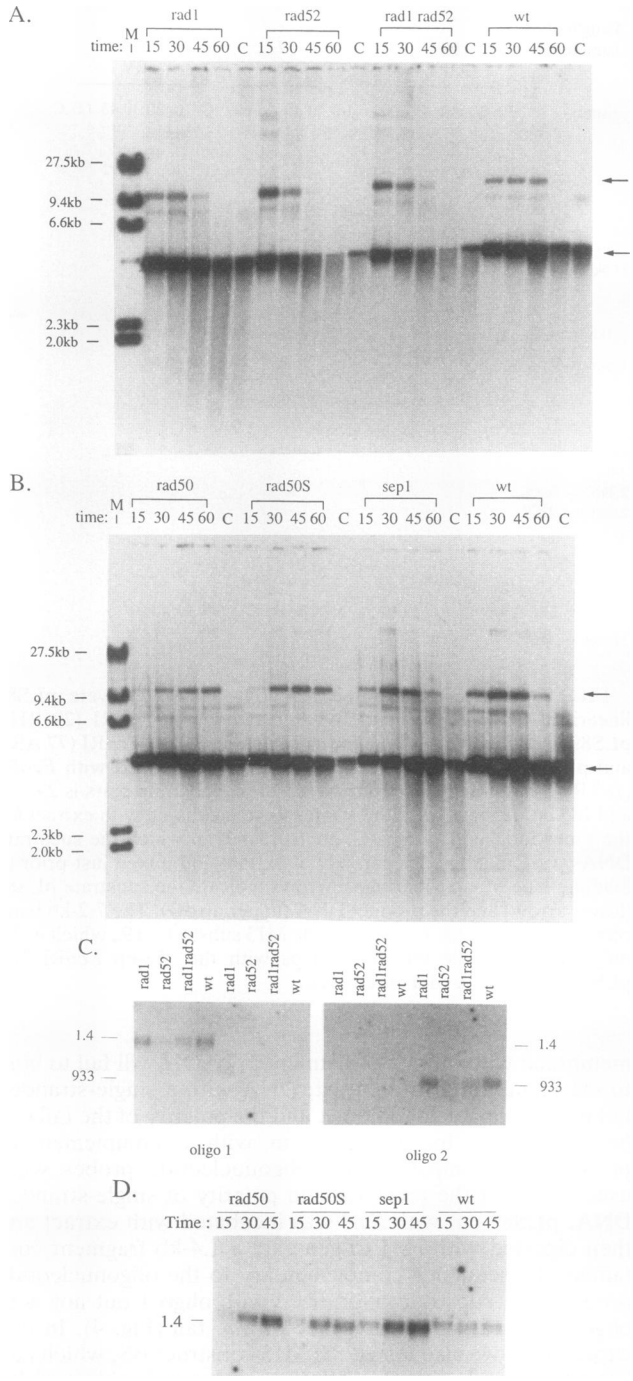


FIG. 6. Analysis of mutant strains. (A and B) Product formation. (A) Extracts from *rad1 rad52*, *rad1 rad52*, and wild-type (wt) strains were assayed for product formation, using the substrate pair pLS89 linearized with *Sal*I and BS linearized with *Bam*HI. Samples were removed from the reaction at the times indicated. (B) Extracts from *rad50*, *rad50S*, *sep1*, and wild-type (wt) strains were assayed with the same substrate pair, and time points were taken as indicated. For both panels A and B, lanes C are controls in which the two substrates were incubated separately in extract and mixed just prior to loading and lanes M contain size markers. (C and D) Exonuclease activity, as assayed by neutral gel transfer. (C) For both blots, extracts from *rad1*, *rad52*, *rad1 rad52*, and wild-type (wt) strains were assayed for exonuclease activity, using neutral gel transfer as described for Fig. 4. In the first four lanes of both blots, the substrate pLS89 linearized with *Sal*I was used for the reaction; in the next four lanes of both blots, the substrate BS linearized with *Bam*HI was used for the reaction. Following incubation with extract for 60 min, the substrate DNA was digested as for Fig. 4. The blots on the left and right were probed with oligo 1 and oligo 2, respectively. (D) Extracts from *rad50*, *rad50S*, *sep1*, and wild-type (wt) strains were assayed by using pLS89 linearized with *Sal*I and taking time points as indicated. The blot was probed with oligo 1.

Fig. 3, the amount of product formed when the substrates were incubated separately in extract and later coprecipitated appeared to be identical to that formed when the two substrates were coincubated in the extract.

The time course of the reaction. A prediction of the SSA model is that substrate pairs with shorter regions of homology should form product faster than do substrates with a longer region of homology. This prediction was tested by using substrates with 29, 77, or 185 bp of terminal homology (Fig. 5). The substrate pairs which share the shortest region of homology, RH with pLS89 and AR with pLS89, were capable of product formation at the earliest time point assayed, 10 min. The third pair, RV with pLS89, did not form product until incubated for at least 20 min in the extract.

Another prediction of the SSA model is that the reaction will be length dependent, with longer substrates requiring additional processing to form product. Our observation that the longest substrates reacted poorly (Fig. 2) is consistent with this prediction. These substrates failed to form product even when incubated for 2 h in the extract (data not shown). It is possible that these substrates define the limit of the exonuclease under our in vitro conditions. Alternatively, the failure of the longest substrates to form product may be due to the significant degradation required for these reactions. If processing occurs on both ends of the DNA substrate simultaneously, degradation sufficient to give product formation will also leave a long single-stranded tail on the nonhomologous end of the substrate, resulting in a product with aberrant mobility and preventing formation of a discrete band.

It was also observed that the product level changed over time, peaking at earlier time points and then decaying (Fig. 5). This rise and fall could be due to the presence of either single-strand-specific exonucleases or endonucleases in the extract which act to degrade the available single-stranded tails prior to annealing. Consistent with this notion is the observation that the RH reaction product disappears earlier than other reaction products. Because this substrate pair shares the least amount of terminal homology, the homologous region would be especially sensitive to degradation.

Analysis of recombination- and repair-defective strains. Nuclear extracts were prepared from a number of strains deficient in recombination or DNA repair and were assayed both for the presence of the exonuclease and for the ability to catalyze product formation. Strains tested include those with mutations in the *RAD1*, *RAD50*, *RAD52*, and *SEP1* genes. Exonuclease activity was present in all mutant strains, including a *rad1 rad52* double mutant (Fig. 6C and D), and all strains were proficient in catalyzing the recombination reaction (Fig. 6A and B).

Cofractionation of an activity that promotes joint molecule formation with a 5′-3′ exonuclease. To confirm that product formation was catalyzed by a 5′-3′ exonuclease, cell extracts were fractionated and assayed for the ability to promote joint molecule formation and for 5′-3′ exonuclease activity. In initial studies using strain LSY150, we identified fractions

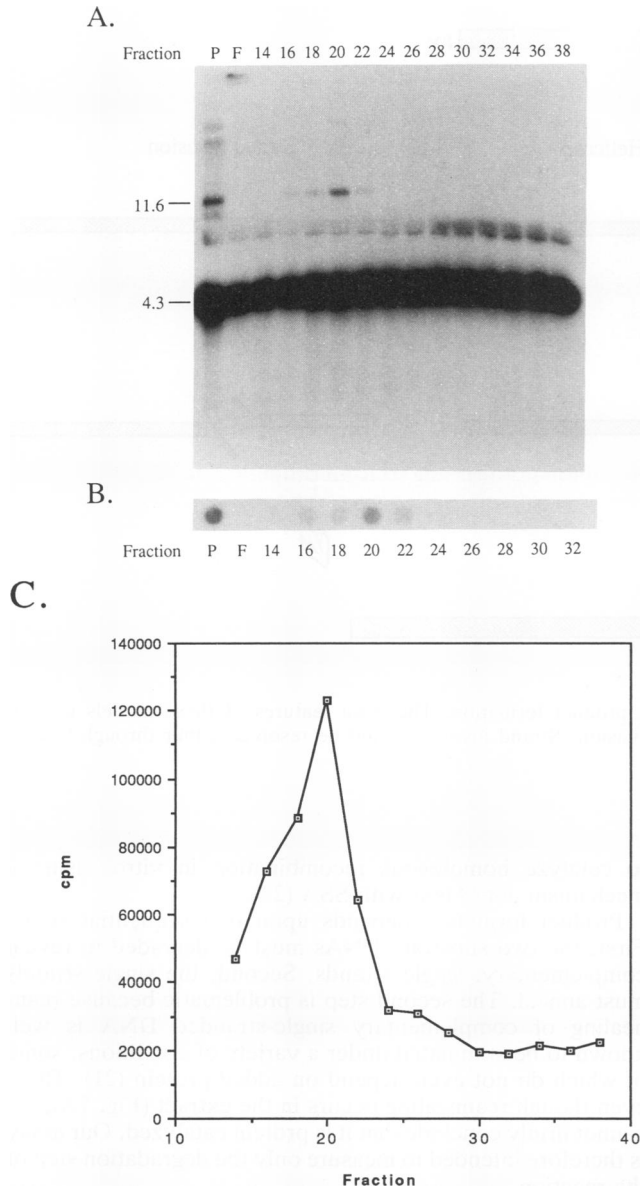


FIG. 7. Assay of column fractions. (A) pLS89 linearized with *SalI* and BS linearized with *BamHI* were incubated with a sample of the loaded protein (P), the column flowthrough (F), or indicated fractions from a heparin agarose column. The two size markers indicate the 11.6-kb product band (upper) and the 4.3-kb substrate band (lower). (B) Dot blot analysis. Protein samples were incubated with pLS89 linearized with *SalI*, transferred to a membrane by using a dot blot apparatus, and probed with oligo 1. (C) Degradation of a uniformly labeled substrate DNA. The graph indicates counts released from ^{32}P-labeled linear pLS89 when incubated with the column fractions indicated.

eluting from DEAE-cellulose which catalyzed joint molecule formation, using the substrate pair with 262 bp of homology. In further purification steps, we attempted to monitor the activity by using only an exonuclease assay which measured release of acid-soluble nucleotides from radiolabeled linear DNA. However, this method identified multiple peaks of activity. We pursued purification of a major exonuclease peak and found that we had enriched for a protein with a molecular size of 175 kDa, corresponding in size to Sep1. This fraction did not catalyze joint molecule formation. This finding indicated the necessity to assay for joint molecule formation as well as for exonuclease activity at all steps in the purification. To eliminate interference by the exonuclease presumably associated with Sep1 and by the mitochondrial exonuclease Nuc1, a strain with deletions in the *SEP1* and *NUC1* genes (LSY346-5A) was constructed. Crude extracts prepared from this strain catalyzed joint molecule formation as efficiently as did wild-type extracts (data not shown).

In addition to the joint molecule formation assay, two assays for exonuclease activity were used to monitor activity. The first was a variation of the neutral gel transfer method in which the reacted pLS89/*SalI* substrate was transferred to a nylon membrane in a dot blot manifold without denaturation. Duplicate filters were then hybridized with oligo 1 or oligo 2 to detect degradation of the 5′ or 3′ end. The second method involved the measurement of liberation of acid-soluble nucleotides from ^{32}P-labeled linear DNA substrates. Whole cell extracts of LSY346-5A were fractionated by salt gradient elution from a DEAE-cellulose column. A single peak of activity that catalyzed joint molecule formation and also contained 5′-3′ exonuclease activity eluted at 44 to 70 mM NaCl. The active fractions were pooled and chromatographed on Superose 12. Again, a single peak of exonuclease activity and joint molecule-forming activity coeluted. These fractions were pooled and chromatographed on heparin agarose. Bound proteins were eluted with a salt gradient, and a single peak of activity that promoted joint molecule formation emerged at 300 to 360 mM NaCl (Fig. 7A). A 5′-3′ exonucleolytic activity as detected by the neutral dot blot assay (Fig. 7B) and by the acid solubilization assay again coeluted (Fig. 7C). On the basis of the protein yield, we estimate that the activity is enriched at least 200-fold by the purification steps described. We conclude that the 5′-3′ exonuclease is necessary for the formation of joint molecules.

DISCUSSION

We have used yeast nuclear extracts to catalyze recombination between two DNA substrates with overlapping, homologous ends. Three models have been proposed to explain this form of recombination (Fig. 8). In the SSA model of Lin et al. (24), a strand-specific exonuclease is proposed to degrade each substrate to leave a single-stranded tail on each. The complementary DNA exposed would then pair to form the observed product. Second, in a variation of this model, a helicase is imagined to unwind the two substrates until the free, complementary strands pair (54). Alternatively, the single-stranded tails produced by a DNA helicase could be acted on by a single-strand-specific exonuclease to generate the same intermediate as that formed by a double-strand-specific exonuclease. In a third model, which is similar to the Meselson-Radding model (33), a DNA end from one substrate participates in strand invasion of the second substrate to form a Holliday structure which is resolved by branch migration or endonucleolytic cleavage to form the observed product.

Our observations are consistent with the first of these three models. First, substrates with larger lengths of homology require increased time in extract to form product (Fig. 5). This is consistent with the action of a processive activity. Product formation requires exposure of a significant portion of homologous DNA on both substrates before pairing can occur. In our model, exposure would occur most rapidly in

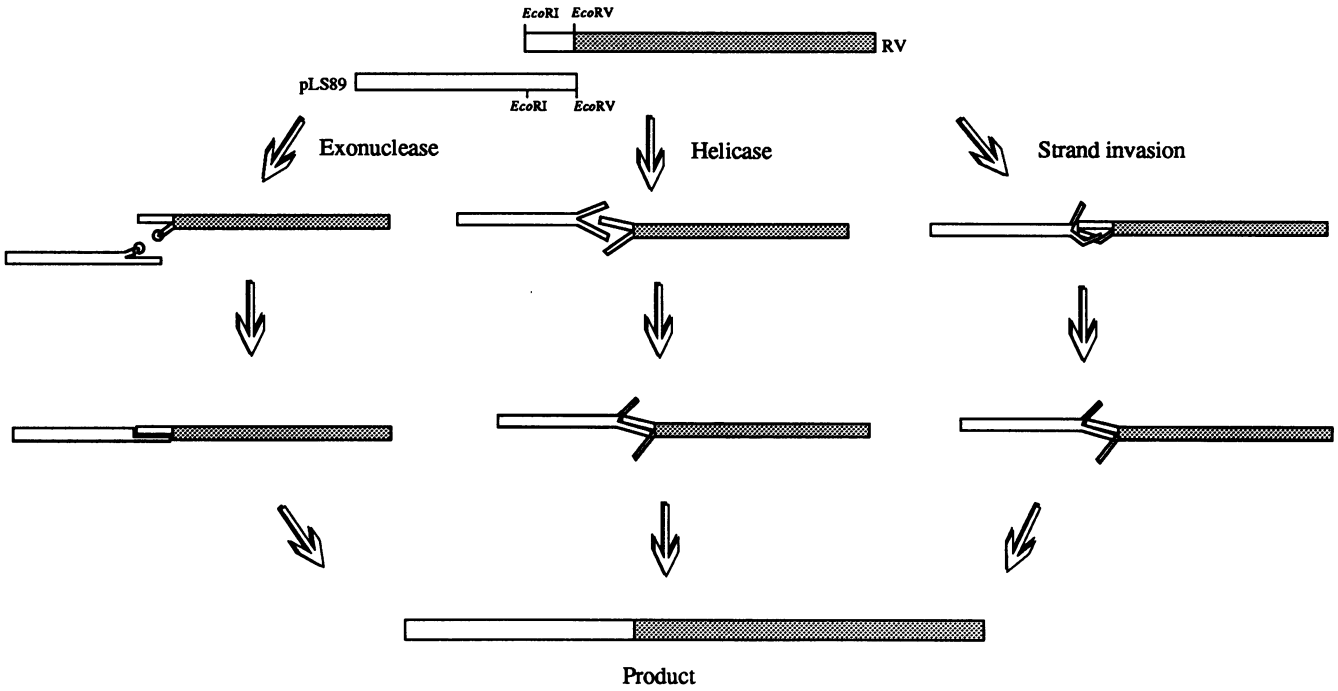


FIG. 8. The reaction. Three models have been proposed to explain product formation. The main features of these models include degradation by an exonuclease, unwinding by a helicase, and strand invasion. Strand invasion could be resolved either through branch migration, as shown above, or through cleavage by an endonuclease.

the shorter substrates and more slowly in longer substrates, resulting in the observed delay. The observation that substrates can be incubated separately with extract and later mixed to form product is also strong evidence for the exonuclease model. Both the helicase and the strand invasion models require participation of both DNA substrates in the presence of the required proteins for product formation. Further evidence against participation by a helicase is our observation that no hydrolyzable nucleotide cofactor is required for the reaction (data not shown). To date, all known DNA helicases require such factors (32). In further support of our model, we have identified a 5′-3′ exonuclease in the extracts which copurifies with the recombination activity monitored in the Southern blot assays.

We propose that this reaction is analogous to reactions which have been described in vivo. Double-strand break-stimulated recombination between sequences in direct repeat, either of *lacZ* on a plasmid (40), of chromosomal *ura3* genes (47), or of chromosomal rDNA copies (35), is capable of proceeding by a nonconservative pathway similar to that used in our assay. In both the in vivo and in vitro situations, it is predicted that strand-specific degradation of two homologs reveals complementary DNA which can anneal to form a single copy of the homologous DNA. Action by a 5′-3′ exonuclease has been demonstrated at two of these loci in vivo (47). It is likely that this pathway for recombination is conserved in many biological systems. In prokaryotes, strand-specific double-stranded exonucleases used for recombination include the λ exonuclease, the T7 gene 6 product, and exonuclease VIII of *E. coli* (41). Maryon and Carroll have similarly identified a 5′-3′ exonuclease in *Xenopus* oocytes which acts on substrate DNA to generate recombination intermediates (29–31). In addition, nuclear extracts prepared from *Xenopus* oocytes have been shown to catalyze homologous recombination in vitro, using a mechanism consistent with SSA (22).

Product formation depends upon two sequential steps. First, the two substrate DNAs must be degraded to reveal complementary, single strands. Second, the single strands must anneal. The second step is problematic because reannealing of complementary single-stranded DNA is well known to be stimulated under a variety of conditions, some of which do not even depend on added protein (21). Thus, even though reannealing occurs in the extract (Fig. 7A), we cannot firmly conclude that it is protein catalyzed. Our assay is therefore intended to measure only the degradation step of the reaction.

To identify the gene products required for this recombination reaction, a number of recombination- and repair-defective strains were assayed. The strains assayed were selected for a variety of reasons. The *RAD52* gene product is required for the repair of double-strand breaks in yeast cells, such that a double-strand break (at loci other than the rDNA [35]) causes lethality in this mutant (27). In addition, most spontaneous recombination is dependent upon the *RAD52* gene product (27, 36). *rad50* mutants are sensitive to X rays and methyl methanesulfonate (MMS) (15). Although spontaneous recombination in a *rad50* mutant is higher than in wild-type cells (1, 28) and integration of linear plasmid DNA occurs normally (1), mating-type switching occurs at a reduced rate and meiotic recombination is abolished (16). *rad50S* is an allele of *RAD50* that differs from the *rad50* null alleles (1) in that it is MMS resistant, and meiosis-specific double-strand breaks are formed but are not processed in this mutant (7, 48). The exonucleolytic processing of a double-strand break in *rad50S* cells during mitotic growth has yet to be determined, but the resistance to MMS suggests that DSBR is not affected during mitotic growth. A

rad1 mutant is UV sensitive and is defective for the integration of linear plasmid DNA (43). The *rad1 rad52* double mutant was selected because of reports that recombination between genes in direct repeat is highly reduced in this background (20, 43, 52), and previous work indicates that such direct repeat constructs may recombine by using the same pathway as do the substrates in our assay (35, 40, 47). *sep1* was chosen because it encodes a strand-exchange protein with an intrinsic 5′-3′ exonuclease activity (19). Because each of these strains was proficient in both product formation and exonuclease activity, we propose that the activity is dependent upon the product of a gene not yet tested by our group.

Of the exonucleases isolated from *S. cerevisiae*, most either are not likely to be involved in the reaction that we observe or are not yet characterized well enough for involvement to be determined. Purified yeast exonucleases include exonuclease II (53) and exonuclease V (6), which are specific for single-stranded DNA. Others include exonuclease IV (3) and Sep1 (19), two 5′-3′ exonucleases which degrade both single-stranded and double-stranded DNA, with a strong preference for the former. The *SEP1* gene product also acts as an exoribonuclease and was independently purified as such (46). Because Sep1 could be responsible for the formation of our observed product, we tested a *sep1* mutant and found that activity was still detected (Fig. 6B and D). Another nuclease to be considered is encoded by the yeast gene *RAT1/HKE1/XRN2*, which shares significant homology with *SEP1* (2). The purified protein has 5′-3′ exoribonuclease activity (19), but it is unknown whether Rat1, like Sep1, has exodeoxyribonuclease activity. Yet another yeast nuclease has been described by Dolberg et al. (12). This 5′-3′ double-stranded exonuclease has weak activity on single-stranded DNA and no detectable activity with RNA. As judged from the reported chromatographic properties, this exonuclease is different from the activity that we have described. Chow and Resnick (9) have identified a nuclease activity that is positively regulated by the *RAD52* gene product. Because the activity observed in our assay is still present, and is perhaps even increased, in a *rad52* mutant, it is unlikely to be related to the activity identified by Chow and Resnick (9). The *NUC1* gene encodes a mitochondrial nuclease which displays 5′-3′ double-stranded exonuclease activity (11). This nuclease is tightly associated with the mitochondrial inner membrane, requiring detergent for both solubilization and activity. Because our protocols do not include such methods, we considered it unlikely that this nuclease was responsible for formation of the product observed in our assay. This conclusion was confirmed by analysis of extracts prepared from a strain containing a deletion of the *NUC1* gene.

Although our assay was designed to simulate the SSA reaction, it is possible that the exonuclease which we observe in our reactions is used for other recombination events in *S. cerevisiae* as well. The DSBR model invokes a strand-specific exonuclease which degrades DNA at the break, leaving a single strand which participates in invasion of the homolog (51). Furthermore, 5′-3′ exonuclease activity has been observed at both the *MAT* (55) and *ARG4* (48) loci during recombination. Whether the exonuclease described in our assay is involved in these reactions remains to be determined.

ACKNOWLEDGMENTS

We thank N. Kleckner, R. Kolodner, and T. Stevens for plasmids and J. McDonald, R. Rothstein, and R. Sternglanz for yeast strains. We also thank A. J. Rattray, R. D. Johnson, and C. S. H. Young for critical reading of the manuscript and for helpful suggestions and A. Stevens for communication of unpublished results.

This work was supported by NIH grant GM41784, by a grant from the Irma T. Hirschl Trust, by a scholarship from the Leukemia Society of America, and in part by a grant from the Lucille P. Markey Charitable Trust. K.H. was supported by NIH predoctoral training grants GM08224 and CA09503.

REFERENCES

1. **Alani, E., R. Padmore, and N. Kleckner.** 1990. Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell **61**:419–436.
2. **Amberg, D. C., A. L. Goldstein, and C. N. Cole.** 1992. Isolation and characterization of *RAT1*: an essential gene of *Saccharomyces cerevisiae* required for the efficient nucleocytoplasmic trafficking of mRNA. Genes Dev. **6**:1173–1189.
3. **Bauer, G. A., H. M. Heller, and P. M. J. Burgers.** 1988. DNA polymerase III from *Saccharomyces cerevisiae*. J. Biol. Chem. **263**:917–924.
4. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
5. **Bishop, D. K., D. Park, L. Xu, and N. Kleckner.** 1992. *DMC1*: a meiosis-specific yeast homolog of *E. coli recA* required for recombination, synaptonemal complex formation, and cell cycle progression. Cell **69**:439–456.
6. **Burgers, P. M. J., G. A. Bauer, and L. Tam.** 1988. Exonuclease V from *Saccharomyces cerevisiae*. J. Biol. Chem. **263**:8099–8105.
7. **Cao, L., E. Alani, and N. Kleckner.** 1990. A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell **61**:1089–1101.
8. **Carroll, D., S. H. Wright, R. K. Wolf, E. Grzesiuk, and E. B. Maryon.** 1986. Efficient homologous recombination of linear DNA substrates after injection into *Xenopus laevis* oocytes. Mol. Cell. Biol. **6**:2053–2061.
9. **Chow, T. Y.-K., and M. A. Resnick.** 1987. Purification and characterization of an endo-exonuclease from *Saccharomyces cerevisiae* that is influenced by the *RAD52* gene. J. Biol. Chem. **262**:17659–17667.
10. **Christman, M. F., F. S. Dietrich, and G. R. Fink.** 1988. Mitotic recombination in the rDNA of *S. cerevisiae* is suppressed by the combined action of DNA topoisomerases I and II. Cell **55**:413–425.
11. **Dake, E., T. J. Hoffman, S. McIntire, A. Hudson, and H. P. Zassenhaus.** 1988. Purification and properties of the major nuclease from mitochondria of *Saccharomyces cerevisiae*. J. Biol. Chem. **263**:7691–7702.
12. **Dolberg, M., C.-P. Baur, and R. Knippers.** 1991. Purification and characterization of a novel 5′ exodeoxyribonuclease from the yeast *Saccharomyces cerevisiae*. Eur. J. Biochem. **198**:783–787.
13. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
14. **Fishman-Lobell, J., N. Rudin, and J. E. Haber.** 1992. Two alternative pathways of double-strand break repair that are kinetically separable and independently modulated. Mol. Cell. Biol. **12**:1292–1303.
15. **Friedberg, E. C.** 1988. Deoxyribonucleic acid repair in the yeast *Saccharomyces cerevisiae*. Microbiol. Rev. **52**:70–102.
16. **Game, J. C., T. J. Zamb, R. J. Braun, M. Resnick, and R. M. Roth.** 1980. The role of radiation (*rad*) genes in meiotic recombination in yeast. Genetics **94**:51–68.
17. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
18. **Johnson, A. W., and R. D. Kolodner.** 1991. Strand exchange protein 1 from *Saccharomyces cerevisiae*. J. Biol. Chem. **266**: 14046–14054.
19. **Kenna, M., A. Stevens, M. McCammon, and M. G. Douglas.** 1993. An essential yeast gene with homology to the exonucle-

ase-encoding *XRN1/KEM1* gene also encodes a protein with exoribonuclease activity. Mol. Cell. Biol. **13**:341–350.
20. **Klein, H. L.** 1988. Different types of recombination events are controlled by the *RAD1* and *RAD52* genes of *Saccharomyces cerevisiae*. Genetics **120**:367–377.
21. **Kohne, D. E., S. A. Levinson, and M. J. Byers.** 1977. Room temperature method for increasing the rate of DNA reassociation by many thousandfold: the phenol emulsion reassociation technique. Biochemistry **16**:5329–5341.
22. **Lehman, C. W., and D. Carroll.** 1991. Homologous recombination catalyzed by a nuclear extract from *Xenopus* oocytes. Proc. Natl. Acad. Sci. USA **88**:10840–10844.
23. **Lichten, M. J., and M. S. Fox.** 1983. Detection of non-homology-containing heteroduplex molecules. Nucleic Acids Res. **11**: 3959–3971.
24. **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.
25. **Lin, F.-L., K. Sperle, and N. Sternberg.** 1990. Intermolecular recombination between DNAs introduced into mouse L cells is mediated by a nonconservative pathway that leads to crossover products. Mol. Cell. Biol. **10**:103–112.
26. **Lin, F.-L., K. Sperle, and N. Sternberg.** 1990. Repair of double-stranded DNA breaks by homologous DNA fragments during transfer of DNA into mouse L cells. Mol. Cell. Biol. **10**:113–119.
27. **Malone, R. E., and R. E. Esposito.** 1980. The *RAD52* gene is required for homothallic interconversion of mating types and spontaneous mitotic recombination in yeast. Proc. Natl. Acad. Sci. USA **77**:503–507.
28. **Malone, R. E., T. Ward, S. Lin, and J. Waring.** 1990. The *RAD50* gene, a member of the double strand break repair epistasis group, is not required for spontaneous mitotic recombination in yeast. Curr. Genet. **18**:111–116.
29. **Maryon, E., and D. Carroll.** 1989. Degradation of linear DNA by a strand-specific exonuclease activity in *Xenopus laevis* oocytes. Mol. Cell. Biol. **9**:4862–4871.
30. **Maryon, E., and D. Carroll.** 1991. Involvement of single-stranded tails in homologous recombination of DNA injected into *Xenopus laevis* oocyte nuclei. Mol. Cell. Biol. **11**:3268–3277.
31. **Maryon, E., and D. Carroll.** 1991. Characterization of recombination intermediates from DNA injected into *Xenopus laevis* oocytes: evidence for a nonconservative mechanism of homologous recombination. Mol. Cell. Biol. **11**:3278–3287.
32. **Matson, S. W., and K. A. Kaiser-Rogers.** 1990. DNA helicases. Annu. Rev. Biochem. **59**:289–329.
33. **Meselson, M. S., and C. M. Radding.** 1975. A general model for genetic recombination. Proc. Natl. Acad. Sci. USA **72**:358–361.
34. **Nicolas, A., D. Treco, N. P. Schultes, and J. W. Szostak.** 1989. An initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae*. Nature (London) **338**:35–39.
35. **Ozenberger, B. A., and G. S. Roeder.** 1991. A unique pathway of double-strand break repair operates in tandemly repeated genes. Mol. Cell. Biol. **11**:1222–1231.
36. **Prakash, S., L. Prakash, W. Burke, and B. A. Montelone.** 1980. Effects of the *RAD52* gene on recombination in *Saccharomyces cerevisiae*. Genetics **94**:31–50.
37. **Richardson, C. C.** 1965. Phosphorylation of nucleic acid by an enzyme from T4 bacteriophage-infected *Escherichia coli*. Proc. Natl. Acad. Sci. USA **54**:158–165.
38. **Rothman, J. H., C. P. Hunter, L. A. Valls, and T. H. Stevens.** 1986. Overproduction-induced mislocalization of a yeast vacuolar protein allows isolation of its structural gene. Proc. Natl. Acad. Sci. USA **83**:3248–3252.
39. **Rothstein, R.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–209.
40. **Rudin, N., E. Sugarman, and J. E. Haber.** 1989. Genetic and physical analysis of double-strand break repair and recombination in *Saccharomyces cerevisiae*. Genetics **122**:519–534.
41. **Sadowski, P. D.** 1985. Role of nucleases in genetic recombination, p. 23–40. *In* S. M. Linn and R. J. Roberts (ed.), Nucleases. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
42. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
43. **Schiestl, R. H., and S. Prakash.** 1988. *RAD1*, an excision repair gene of *Saccharomyces cerevisiae*, is also involved in recombination. Mol. Cell. Biol. **8**:3619–3626.
44. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
45. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
46. **Stevens, A.** 1980. Purification and characterization of a *Saccharomyces cerevisiae* exoribonuclease which yields 5′-mononucleotides by a 5′-3′ mode of hydrolysis. J. Biol. Chem. **255**:3080–3085.
47. **Sugawara, N., and J. E. Haber.** Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. Mol. Cell. Biol. **12**:563–575.
48. **Sun, H., D. Treco, and J. W. Szostak.** 1991. Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the *ARG4* recombination initiation site. Cell **64**:1155–1161.
49. **Symington, L. S.** 1991. Double-strand-break repair and recombination catalyzed by a nuclear extract of *Saccharomyces cerevisiae*. EMBO J. **10**:987–996.
50. **Symington, L. S., L. M. Fogarty, and R. D. Kolodner.** 1983. Genetic recombination of homologous plasmids catalyzed by cell-free extracts of *Saccharomyces cerevisiae*. Cell **35**:805–813.
51. **Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein, and F. W. Stahl.** 1983. The double-strand-break repair model for recombination. Cell **33**:25–35.
52. **Thomas, B. J., and R. Rothstein.** 1989. The genetic control of direct-repeat recombination in *Saccharomyces*: the effect of *rad52* and *rad1* on mitotic recombination at *GAL10*, a transcriptionally regulated gene. Genetics **123**:725–738.
53. **Villadsen, I. S., S. E. Bjorn, and A. Vrang.** 1982. Exonuclease II from *Saccharomyces cerevisiae*. J. Biol. Chem. **257**:8177–8182.
54. **Wake, C. T., F. Vernaleone, and J. H. Wilson.** 1985. Topological requirements for homologous recombination among DNA molecules transfected into mammalian cells. Mol. Cell. Biol. **5**:2080–2089.
55. **White, C. I., and J. E. Haber.** 1990. Intermediates of recombination during mating type switching in *Saccharomyces cerevisiae*. EMBO J. **9**:663–673.
56. **Wootner, M., P. A. Wade, J. Bonner, and J. A. Jaehning.** 1991. Transcriptional activation in an improved whole-cell extract from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11**:4555–4560.