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ATP-independent DNA strand transfer catalyzed by protein(s) from meiotic cells of the yeast *Saccharomyces cerevisiae*

(strand exchange/yeast single-stranded DNA binding proteins/meiosis/recombination)

AKIO SUGINO*†, JOHN NITISS‡§, AND MICHAEL A. RESNICK‡

*Laboratory of Genetics and ‡Cellular and Genetic Toxicology Branch, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709

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ABSTRACT **An activity that catalyzes the transfer of a strand from a duplex linear molecule of DNA to a complementary circular single strand can be detected in crude extracts from mitotic and meiotic cells of the yeast *Saccharomyces cerevisiae* by adding yeast single-stranded DNA binding proteins. This DNA strand-transfer activity increases >15-fold during meiosis in *MAT*a/*MAT*α diploids prior to the detection of a 100- to 1000-fold increase in homologous chromosomal recombination. No increase is observed in *MAT*a/*MAT*a or *MAT*α/*MAT*α cells, which do not undergo meiosis when shifted to meiotic medium, suggesting the activity is related to meiotic recombination. The activity is named strand-transfer protein α (STPα) and has been extensively purified from the meiotic cells (6 hr after exposure to sporulation medium). The apparent molecular mass of STPα is 38 kDa under denaturing conditions. The DNA strand-transfer reaction catalyzed by STPα requires homologous single-stranded and double-stranded DNA and Mg^{2+} but no nucleotide cofactor. Yeast single-stranded DNA binding proteins stimulate the reaction at least 10-fold. Among the products analyzed by electron microscopy were typical strand-exchange structures.**

Several types of genetic recombination have been identified in the yeast *Saccharomyces cerevisiae* (see ref. 1 for review). Many genes have been shown to be involved in the various types of recombination and some of these might be expected to code for products that catalyze reactions that might involve recombination processes. It has been well established that, during meiosis, intragenic recombination between homologous chromosomes can increase 100- to 1000-fold in comparison with that during mitosis. The *rad50* and *spo11* mutants block initial steps in meiotic recombination, whereas mutations in the *RAD52*, *-54*, and *-57* genes appear to block intermediate stages (see ref. 2 for review).

To date, it has been possible to identify DNA changes associated with recombination (3, 4) and to characterize some enzymes that may be involved (5–7). Based on studies with several organisms and human cells (see ref. 8 for review), an important category of proteins, the strand-exchange proteins, is expected to be involved in recombination. Though their properties vary, they are able to facilitate DNA–DNA interactions *in vitro*.

Since single-stranded DNA (ssDNA) binding proteins from *Escherichia coli* and bacteriophage T4 (*E. coli* SSB and gene *32* protein, respectively) play important roles in recombination-related reactions *in vitro* as well as *in vivo* (see ref. 8 for review), we expected that some of the SSBs in yeast cells could also be required for recombination. Recently, we purified several immunologically distinct ssDNA binding proteins from the yeast *S. cerevisiae* (ySSBs) (14, 20, 26, 35, and 42 kDa) (R. Hamatake and A.S., unpublished data). Therefore, when looking for activities in extracts that would catalyze reactions presumed to be involved in recombination, ySSBs were included.

In this report, we describe an activity in extracts of meiotic yeast cells that catalyzes the transfer of a strand from a double-stranded DNA (dsDNA) to a complementary circular single strand in the presence of ySSB.

MATERIALS AND METHODS

DNAs. φX174 viral and replicative form I and II DNAs (RF-I and RF-II DNAs) were purchased from Bethesda Research Laboratories. M13mp19 viral and RF-I DNAs were prepared as published (9). ^{32}P-labeled φX174 RF-III DNAs were prepared as follows. For 3′ end-labeled DNA, 100 μg of φX174 RF-I DNA was digested with 200 units of *Xho* I. The 4-base-pair sticky ends were filled in with three dNTPs and [α-^{32}P]dATP (specific activity, 400 Ci/mmol; 1 Ci = 37 GBq) using 2 units of *E. coli* DNA polymerase I large fragment at 4°C for 20 min. The labeled DNA was purified by a Sephadex G-100 gel column, treated twice with phenol, precipitated with ethanol, and dissolved in 10 mM Tris·HCl, pH 7.5/1 mM EDTA. For 5′ labeled DNA, 100 μg of φX174 RF-I DNA was digested with 200 units of *Stu* I. The 5′-phosphate was removed by alkaline phosphatase and 5′ ends were labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. The resultant radioactive DNA was purified as above. Nonradioactive φX174 RF-III DNA was prepared as for the radioactive substrate except that the DNA was restricted with *Stu* I. M13mp19 RF-III DNA was prepared in a similar manner as φX174 RF-III DNA using *Sma* I restriction enzyme. Such blunt-end substrates are able to reduce the possibility that contaminating enzyme(s) would form RF-II DNA.

Yeast Strains. Yeast strains used were *S. cerevisiae* MR48 (*MAT*a/*MAT*α *leu2/leu2 rad52-1/+ rad1-1/+ +/tup7 ade2-1/+ can1/+ hom3-10/+ his1-7/his1-1 +/trp2 +/lys1-1*) (5) and its *MAT*a/*MAT*a and *MAT*α/*MAT*α derivatives (5); all are *S. cerevisiae* SK-1 derivatives.

Other Materials. ssDNA cellulose was made as published (10). Other materials used were described (11). [α-^{32}P]dATP (400 Ci/mmol) and [γ-^{32}P]ATP (3000 Ci/mmol) were from Amersham.

Growth and Recombination. Conditions for growth and sporulation of yeast strains have been described (5). Cells were collected by centrifugation, resuspended in 10% sucrose/50 mM Tris·HCl, pH 7.5/10 mM EDTA, and frozen in liquid nitrogen (5). Sporulation was checked with a phase-contract microscope. Recombination was monitored

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Abbreviations: RF, replicative form; ss, single-stranded; ds, double-stranded; SSB, single-stranded DNA binding protein; ySSB, yeast SSB; STPα, strand-transfer protein α.

†To whom reprint requests should be addressed.
§Present address: Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.

by the appearance of histidine prototrophs arising between *his1-1* and *his1-7* heteroalleles (5).

Enzymes. *E. coli* DNA polymerase I large fragment (Klenow), T4 polynucleotide kinase, *E. coli* alkaline phosphatase, and restriction endonucleases were purchased from Bethesda Research Laboratories. Bacteriophage T4 gene *32* protein was a gift from David C. Mace. *E. coli* SSB was from Worthington. The 14-, 20-, 26-, 35-, and 42-kDa ySSBs were purified from *S. cerevisiae* CB001 by using the nitrocellulose binding assay and yeast DNA polymerase I stimulation assay; their purities were >95% based upon analysis by polyacrylamide gel electrophoresis under denaturing conditions (Fig. 2; R. Hamatake and A.S., unpublished data). No detectable DNase activity was found in these preparations by using the same assay conditions as was used for the strand-transfer protein (see below).

DNA Strand-Transfer Assay. The standard reaction mixture (0.025 ml) contained 50 mM Tris·HCl; (pH 8.1), 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mg of bovine serum albumin per ml, 0.48 μg of ϕX174 viral ssDNA, 0.25 μg of ϕX174 RF-III DNA, yeast protein and 4.8 μg of 20-kDa ySSB. After incubation at 30°C for 15–30 min, 0.005 ml of the stop solution (5% $NaDodSO_4$/25% glycerol/0.5% bromophenol blue) was added, and the total sample was loaded onto 1% agarose gels in TBE buffer (12) and electrophoresed at room temperature at 4 V/cm for 5–16 hr. Occasionally, the reactions were terminated by addition of 10 mM EDTA/1% $NaDodSO_4$ and the products were treated with 0.1 mg of proteinase K per ml at 25°C for 30 min; this was followed by phenol extraction and ethanol precipitation (12). However, the gel pattern of the product was virtually the same as above. To quantitate the formation of RF-II DNA and other joint molecules, the gels were stained with ethidium bromide, photographed (12), and dried. The region of RF-II and RF-III DNAs as well as the remaining portion of the gel were cut out and their radioactivities were measured. In the case of nonradioactive substrates, the negatives of the photograph were scanned by a densitometer (12). Due to the complexity of the reaction products, we tentatively define 1 unit of strand-transfer activity as the activity that results in 25 fmol (≈0.1 μg) of ϕX174 RF-III DNA being used for the reaction in 15 min at 30°C in the presence of ySSB.

Purification of a Yeast Strand-Transfer Protein. All operations were carried out at 0–4°C. Phenylmethylsulfonyl fluoride (1 mM) was added to all solutions. The cell suspension was incubated with 1 mg of Zymolyase 100T per ml in the presence of 10 mM 2-mercaptoethanol for 60 min at 0°C. The cells were collected by centrifugation at 5000 rpm for 5 min in a Sorvall GSA rotor, resuspended in the same volume of 1 M NaCl/50 mM Tris·HCl, pH 7.5/10 mM EDTA/10 mM 2-mercaptoethanol, and incubated at 0°C for another 60 min. The cell lysate was centrifuged at 30,000 rpm for 30 min at 4°C, and the supernatant (crude extract) was saved. To the supernatant, 0.40 g of solid $(NH_4)_2SO_4$ was added per ml, and the precipitate was collected by centrifugation at 10,000 × *g* for 20 min. The precipitate was suspended in buffer A (50 mM Tris·HCl, pH 7.5/10% glycerol/1 mM EDTA/10 mM 2-mercaptoethanol) and dialyzed against the same buffer for 8 hr (fraction I). Fraction I was then applied to a ssDNA cellulose column (50 ml) equilibrated with buffer A containing 50 mM NaCl, and the proteins were eluted with a 500-ml linear gradient from 0.05 to 0.7 M NaCl in buffer A. Active fractions (0.2–0.4 M NaCl) were pooled, dialyzed against buffer A for 3 hr (fraction II), and applied to 5 ml of phosphocellulose (Bio-Rad, Cellex P) equilibrated with buffer A. The activity was eluted with a 50-ml linear gradient from 0 to 0.7 M NaCl in buffer A. The active fractions (0.4–0.6 M NaCl) were pooled (fraction III) and directly applied to a 2-ml hydroxylapatite column equilibrated with buffer A. The activity was eluted with a 50-ml linear gradient from 0 to 0.5 M KPO_4 buffer, pH 7.0/10% glycerol/10 mM 2-mercaptoethanol. The active fractions (0.2–0.3 M KPO_4 buffer) were pooled (fraction IV). Fraction IV was then applied to a 1-ml heparin-Sepharose column equilibrated with buffer A containing 0.1 M NaCl, and the activity was eluted with a 25-ml linear gradient from 0.1 to 1 M NaCl in buffer A. The active fractions (0.4–0.5 M NaCl) were pooled (fraction V). The sample was finally applied on a 1.8 × 90 cm Sephacryl S-300 column equilibrated with 0.15 M NaCl in buffer A, and the activity was eluted with the same buffer. The active fractions were pooled, concentrated, dialyzed against 50% glycerol/buffer A/50 mM NaCl, and stored at −20°C (fraction VI). All experiments to characterize the biochemical properties of strand-transfer protein α (STPα) were carried out with fraction VI.

Other Methods. Agarose and polyacrylamide gel electrophoreses were carried out as described (12). DNA-dependent and DNA-independent ATPase activities were measured as described (11). Electron microscopic examination of the products by STPα was carried out as described (13).

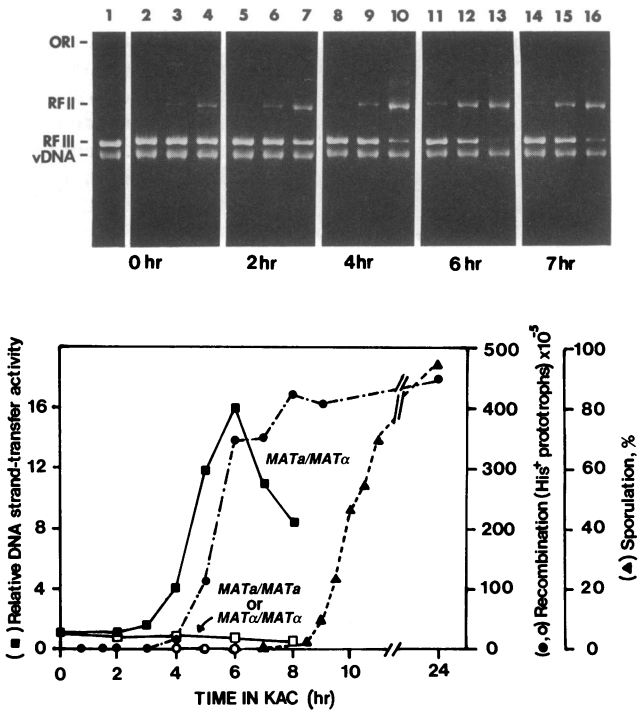

FIG. 1. DNA strand-transfer activity induced during meiosis in yeast. *S. cerevisiae* MR48 (*MATa*/*MATα*), aa3 (*MATa*/*MATa*), or αα3 (*MATα*/*MATα*) cells were grown in 1 liter of SPS medium to 4 × 10^7 per ml, harvested, resuspended to 5 × 10^7 per ml, and incubated in acetate medium. At various times of incubation, 100-ml samples were withdrawn, the cells were lysed, and fraction I was prepared and assayed for strand-transfer activity by using unlabeled substrates. (*Upper*) Representative agarose gel assay of the strand-transfer activity in fraction I from either cells harvested prior to or after changing from SPS to acetate medium. The assays were carried out in the presence of 20-kDa ySSB. Lane 1, substrate with ySSB. Lanes 2–16, reaction products using fraction I from mitotic cells (0 hr) (lanes 2–4) or from cells incubated for 2 hr (lanes 5–7), 4 hr (lanes 8–10), 6 hr (lanes 11–13), or 7 hr (lanes 14–16) in acetate medium (meiotic cells). Lanes 2, 5, 8, 11, and 14 contained 0.2 μg of protein; lanes 3, 6, 9, 12, and 15 contained 1.0 μg of protein; and lanes 4, 7, 10, 13, and 16 contained 5 μg of protein. vDNA indicates the position of ϕX174 viral ssDNA. ORI, origin. (*Lower*) Time course of DNA strand-transfer activity, intragenic recombination (appearance of His^+ prototrophs) relative to time t = 0 hr, and sporulation during meiosis in *MATa*/*MATα* (closed symbols), *MATa*/*MATa*, or *MATα*/*MATα* cells; to simplify the figure, only *MATα*/*MATα* is shown (open symbols). Strand-transfer activity during meiosis was normalized to the activity in fraction I from mitotic cells.

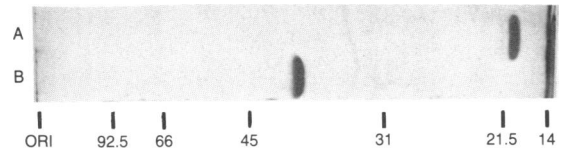

FIG. 2. Purity of STPα and 20-kDa ySSB. STPα (fraction VI, 2 μg), 20-kDa ySSB (2 μg), and marker proteins were subjected to 10% polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$, and the gel was stained with silver nitrate. Molecular masses are given in kDa. Lane A, 20-kDa ySSB; lane B, STPα. ORI, origin.

RESULTS

Strand-Transfer Activity in Crude Extracts Increases Concomitantly with Intragenic Recombination During Meiosis. Meiosis in *S. cerevisiae* increases intragenic recombination between homologous chromosomes by as much as 100- to 1000-fold over that in mitotic cells. Since some enzymes and factors required for recombination are likely to be induced during meiosis, we looked for an activity that catalyzes strand exchange between homologous linear dsDNA and circular ssDNA (8) in meiotic yeast cells. Crude extracts were prepared from diploid cells harvested at various times after switching from vegetative growth in SPS medium to sporulation medium (KAC) (5). They were examined for an activity able to generate the product that comigrates with RF-II DNA from φX174 RF-III and viral DNA in the presence of 20-kDa ySSB. As shown in Fig. 1 *Upper* and *Lower*, the activity in wild-type *MATa*/*MATα* diploid cells increased at least 15-fold within 6 hr after the cells were switched to sporulation medium. This increase was related to the increased commitment to recombination observed as cells proceeded through meiosis. The increased activity started ≈1 hr prior to the large increase of His^+ prototrophs. On the other hand, *MATa*/*MATa* or *MATα*/*MATα* cells, which do not undergo meiotic development, exhibited increases in neither strand-transfer activity nor recombination (Fig. 1 *Lower*). Following an additional step of purification (phosphocellulose column chromatography), results similar to those shown in Fig. 1 were obtained (data not shown). The at least 15-fold increase in activity observed was not simply due to an increase in ySSB, since the amount of ySSB in crude extracts (probed by antibodies against 14-, 20-, 35-, and 42-kDa ySSB) was approximately constant throughout meiosis (data not shown).

Purification of DNA Strand-Transfer Activity from Meiotic Cells. To prove that the products formed by meiotic crude extracts are in fact DNA strand-exchange products, the activity was extensively purified from extracts of meiotic cells (MR48) incubated for 6 hr in sporulation medium. The activity was measured in the presence of 20-kDa yeast SSB (Fig. 2) throughout purification (Table 1). $NaDodSO_4$/polyacrylamide gel electrophoresis of fraction VI showed one major polypeptide of 38 kDa that coincided with the activity (Fig. 2, lane 1). Therefore, we concluded that the 38-kDa polypeptide catalyzes the DNA strand-transfer reaction and we tentatively name it yeast strand-transfer protein α (STPα). Neither DNA-dependent nor DNA-independent ATPase activity could be detected in the active fractions (data not shown).

DNA Strand-Transfer Reaction Catalyzed by STPα Is Stimulated by ySSBs. Strand-transfer activity can be detected in crude extracts from meiotic and mitotic *S. cerevisiae* cells without exogenously added ySSB. However, after one further purification step, activity was hardly detectable without ySSB, presumably due to the removal of endogenous ySSBs and/or dilution of the protein(s). We investigated the ySSB requirement for the strand-transfer reaction catalyzed by purified STPα. As shown in Fig. 3, the 20-kDa ySSB stimulated the STPα-catalyzed reaction at least 10-fold. The majority of the product in the presence of ySSB comigrated with the RF-II DNA (see Fig. 4), whereas the main product made by STPα alone was not the complete strand-exchange structure and no RF-II or RF-II-like DNA was detected. The concentration of ySSB is critical: the optimum is one ySSB molecule per 6–8 nucleotides of ssDNA. At more than one molecule per 6–8 nucleotides of ssDNA, DNA strand exchange was markedly reduced. The reaction stimulated by ySSB does not appear to depend on the concentration of linear dsDNA. When five times more φX174 RF-III DNA than that of Fig. 3 was used, the optimum was the same as observed in Fig. 3. Three other ySSBs (14, 26, and 35 kDa) stimulated the activity as much as 20-kDa ySSB. The *E. coli* SSB, T4 gene *32* protein, and 42-kDa ySSB stimulated the activity only 2-fold (data not shown).

General Properties of the Reaction Catalyzed by STPα. DNA strand transfer catalyzed by STPα and ySSB absolutely requires homologous ds- and ssDNAs [for example, φX174 vs. φX174 (Fig. 4, lanes 6 and 7) or M13mp19 vs. M13mp19 (not shown)] and Mg^{2+}. The ratio between RF-III DNA and circular ssDNA (from 0.25 to 2) did not change significantly the activity. Most of the product comigrates with φX174 RF-II DNA. To further ascertain that the reaction products are the structures expected from DNA strand transfer, the product that comigrated with the RF-II DNA was eluted from the gel and was examined with an electron microscope. Fifty-three percent of the observed DNA molecules (54/102) was fully relaxed, circular dsDNA (Fig. 5*A*), 31% was a typical intermediate structure (32/102, Fig. 5 *B* and *C*), and the remaining 16% was the more complicated structures (16/102, not shown). No ATP or other nucleotides are required for the activity (Fig. 4). Instead, ATP, ATP[γS] and ADP inhibited the activity by 30–40%. The activity is salt sensitive: 100 and 200 mM NaCl inhibited it by >80% and >95%, respectively. The optimal pH was between 7.5 and 8.5 and the optimal temperature was between 35°C and 37°C. Any obvious reaction lag could not be observed and the reaction was very fast (Fig. 4, lanes 12 and 13). We detected neither endo- nor exodeoxyribonuclease activity in the most purified fraction (fraction VI). Absence of endodeoxyribo-

Table 1. Purification of STPα

Fraction	Total volume, ml	Total protein, mg	Total activity, units	Specific activity, units/mg	Yield, %	Purification, fold
Crude extract	1000	3000	ND	ND	—	—
$(NH_4)_2SO_4$ (I)	220	1760	1.37×10^5	79	100	1
ssDNA cellulose (II)	120	68	1.25×10^5	1,840	91	23
Cellex P (III)	38	7.2	6.62×10^4	9,100	48	117
Hydroxylapatite (IV)	30	2.4	4.37×10^4	18,300	32	233
Heparin-Sepharose (V)	10	0.50	3.50×10^4	70,000	26	896
Sephacryl S-300 (VI)	2	0.15	2.37×10^4	159,000	17	2030

Cells (1.6×10^{12}) (from 40 liters) harvested 6 hr after incubation in meiotic medium were used. ND, not determined.

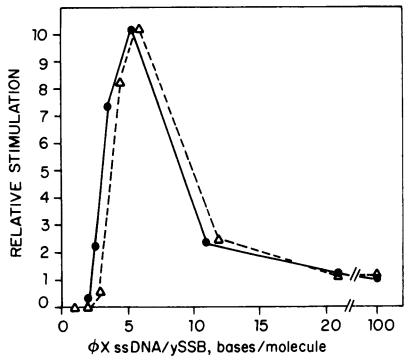

FIG. 3. Stimulation of the STPα activity by 20-kDa ySSB. The reaction mixtures contained 50 ng of STPα, either 120 (△) or 240 (●) ng of ϕX174 viral ssDNA, 250 ng of 3′ ^{32}P-labeled ϕX174 RF-III DNA, and various amounts of 20-kDa ySSB. They were incubated at 30°C for 15 min and the products were analyzed by electrophoresis in a 1% agarose gel. After staining with ethidium bromide and drying the gel, the remaining ϕX174 RF-III was identified and its radioactivity was measured. The activity in the presence of ySSB was normalized to the activity without ySSB.

nuclease was determined by failure to observe any change in the electrophoretic properties of the ϕX174 ssDNA and RF-I DNA (0.25 μg) used to assay the activity following incubation at 37°C for 30 min with 0.2 μg of fraction VI. Absence of exonuclease was determined by failure to detect any loss of ^{32}P label (<0.3 fmol) at the 3′ or 5′ end of the native or heat-denatured ϕX174 RF-III (ends, 64 fmol; specific activity, 470 cpm/fmol) used in the Fig. 4 following incubation at 37°C for 30 min with 0.2 μg of fractions VI.

Relatively little displaced linear ssDNA was seen in this reaction (Fig. 4, lanes 6, 7, 12, and 13), although typical RF-II-like molecules could be observed (Fig. 5*A*) in the band that comigrated with marker RF-II. The inability to detect the

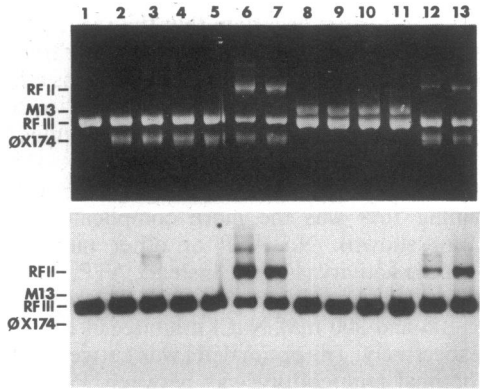

FIG. 4. Requirements for DNA strand transfer catalyzed by STPα. The complete reaction mixtures contained 250 ng of 3′ ^{32}P-labeled αX174 RF-III DNA, 480 ng of ϕX174 viral DNA, 4.8 μg of 20-kDa ySSB, and 40 ng of STPα and were incubated at 37°C for 15 min; the products were analyzed as for Fig. 1 *Upper*. The upper panel shows a photograph of the gel and the lower panel shows an autoradiograph of the dried gel. Lane 1, ϕX174 RF-III alone (no enzyme); lane 2, as lane 1 but with ϕX174 viral ssDNA (no enzyme); lane 3, as lane 2 but with STPα; lane 4, complete mixture without $MgCl_2$; lane 5, complete mixture without STPα; lane 6, complete mixture; lane 7, complete mixture with 1 mM ATP; lane 8, M13mp19 viral ssDNA was added instead of ϕX174 viral DNA (no enzyme); lane 9, as lane 8 but with the STPα; lane 10, as lane 9 but with ySSB; lane 11, as lane 10 but with 1 mM ATP; lane 12, as lane 6 but the incubation was for 0.5 min; lane 13, as lane 6 but incubation was for 2 min. RF II, RF III, ϕX174, and M13, positions of ϕX174 RF-II, ϕX174 RF-III, ϕX174 viral ssDNA, and M13mp19 viral ssDNA, respectively. A similar experiment was carried out with 5′ end-labeled ϕX174 RF-III and the result was virtually the same as that of 3′ end-labeled substrate.

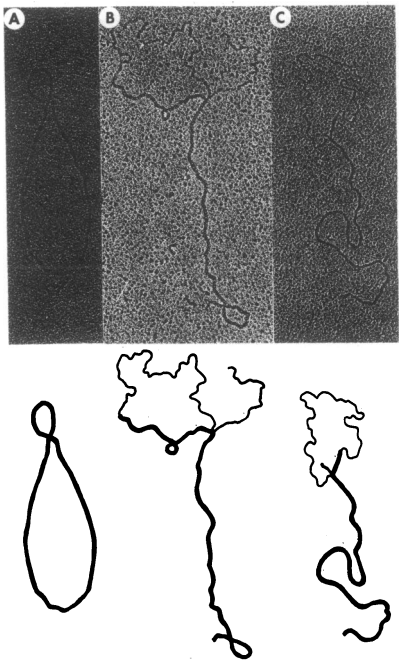

FIG. 5. Electron microscopic picture of the products made by STPα and 20-kDa ySSB. The reaction was carried out under the same condition as for lane 6 of Fig. 4 with unlabeled ϕX174 RF-III and was electrophoresed in a 1% low-melting agarose gel at 4°C. The DNA that comigrated with the RF-II was extracted from the gel and treated with cytochrome *c*. After shadowing, the DNA was examined by an electron microscope. Representative DNA molecules are shown. The lower drawings show interpretation of each picture. The thick line represents a dsDNA and the thin line is a ssDNA. (*A*) Open circular dsDNA. (*B* and *C*) Joint molecules.

expected linear ssDNA could not be explained by degradation since the total radioactivity associated with the linear dsDNA (RF-III) was not lost after strand transfer (Fig. 4 and Table 2). The displaced strands probably invade other RF-II and/or RF-III DNA molecules to form more complex structures. Similar phenomena have been reported for bacteriophage T4 UvsX protein (14).

DISCUSSION

We have identified and characterized an activity from crude extracts of meiotic yeast cells that catalyzes strand transfer

Table 2. Radioactivity at ends of a linear DNA molecule is not lost after a reaction by STPα

Lane in Fig. 4	Radioactivity, cpm				
	RF-III	RF-II*	Other region†	Total	%
1	30,782	0	0	30,882	100
2	30,598	0	0	30,598	99
3	30,385	0	395	30,780	100
4	30,408	0	128	30,780	100
5	30,513	182	119	30,812	100
6	9,743	19,490	2,085	31,318	102
7	15,564	14,847	435	30,846	100
8	30,985	0	0	30,985	101
9	31,098	0	0	31,098	101
10	30,881	0	121	31,002	100
11	30,659	0	209	30,868	100
12	28,956	1,498	119	30,573	99
13	26,852	3,956	201	31,009	101

*The region where marker RF-II DNA migrated was designated as RF-II. Thus, it does not necessarily represent true RF-II DNA.
†After cutting regions where ϕX174 RF-II and RF-III migrated from the gel, the rest of the gel above RF-III was used for the measurement.

between homologous dsDNAs and circular ssDNAs in the presence of ySSBs. Crude extracts do not show potent exo- and endonuclease activities in either the presence or absence of exogenously added ySSB; the unreacted dsDNA and ssDNA substrates were still intact and the products of DNA strand exchange (RF-II and/or RF-II-like molecules) could be readily detected (Fig. 1). Although it is time-consuming and less sensitive than other assays, our assay is more reliable than the nitrocellulose filter-binding and S1 nuclease assays (15). Since the activity was subsequently shown not to require ATP, a DNA-dependent ATPase assay could not be used. After a >2000-fold purification, sufficiently pure protein was obtained to characterize the reaction (Fig. 2 and Table 1). One major 38-kDa polypeptide was detected by denatured polyacrylamide gel electrophoresis (Fig. 2) and was >90% pure, assuming its polypeptide is responsible for the activity.

The reaction catalyzed by fraction VI (Table I) in the presence or absence of ySSB requires no nucleotide cofactors. On the contrary, ATP is inhibitory (Fig. 4, lane 7). Fraction VI contains no detectable DNA-dependent or DNA-independent ATPase activity. Several observations eliminate more trivial explanations for this ATP-independent reaction. (*i*) The purified protein lacks detectable exo- and endonuclease activities and DNA topoisomerase activity, any of which might facilitate the reaction. (*ii*) The total radioactivity in either 3′ or 5′ end-labeled RF-III DNA molecule was conserved during the reaction (Fig. 4 and Table 2, and not shown in the case of 5′ end-labeled substrate). Therefore, it is unlikely that contaminating exonuclease activity generates sticky ends capable of generating RF-II-like molecules by spontaneous annealing. (*iii*) The reaction absolutely requires homologous ssDNA (Fig. 4). (*iv*) Roughly the same amount of ssDNA was consumed as the products appeared (Figs. 1 and 4). (*v*) The reaction products are the expected structures from strand-exchange reaction—namely, the length of the displaced single strand is the same as that of the double strand seen on the circular DNA molecule and open circular dsDNA (Fig. 5).

It is unexpected that the STPα can detect homology and promote strand exchange (which involves unwinding of dsDNA) without a nucleotide energy source, since similar proteins described from prokaryotes and eukaryotes (see ref. 8 for review) require ATP for strand exchange. Nonetheless, the strand-transfer reaction is efficient, extensive, and occurs rapidly with the help of ySSB (Fig. 4). Of the three likely steps in strand transfer—DNA aggregation (synapsis), homology recognition at ends of molecules, and branch migration—it is believed that extensive branch migration requires net energy (16). This assumes that the reaction pathway does not require unwinding of duplex DNA molecules involved in the reaction. The amount of energy required for directed branch migration is not known, but it could be small, since the free energy change of undirected branch migration is zero. The required energy could be partly provided by the interaction of the ySSB and DNA. Since 1 mol of 20-kDa ySSB per 6–8 bases of ssDNA is required for optimal conditions, the energetics of this interaction may be important in obtaining efficient branch migration. ySSB and STPα could promote interaction between dsDNAs and ssDNAs and could facilitate a search for homology. Once homology between ends was established, strand exchange could occur by branch migration provided that no mismatches are present (16).

So far, six different proteins catalyzing DNA strand exchange and other reactions perhaps related to recombination have been described in various systems (8). With the single exception of a human recombinase (17), which catalyzes limited strand exchange in an ATP-independent manner, they require stoichiometric amounts of protein for strand exchange even in the presence of SSB. It has been well established that the reaction catalyzed by these proteins absolutely requires ATP hydrolysis (8). Therefore, the yeast protein described here is a unique kind of strand-exchange protein.

Several observations support, but do not provide direct evidence for, the conclusion that ySTPα is required for recombination. The activity increases considerably in *MAT***a**/*MAT*α diploid cells during meiosis, ≈1 hr prior to the large increase of meiotic intragenic recombination between homologous chromosomes (Fig. 1). Soon after commitment to recombination reaches a maximum, the strand-transfer activity starts decreasing (Fig. 1). We have also examined the activity in mutants that are defective in meiotic recombination (18). Although the activity is detected in mitotically growing *rad50* cells, there is no increase during meiosis. *rad50* mutants can undergo meiosis; however, no increase in recombination between homologous chromosome is detected during meiosis (2). The small amount of STPα required for *in vitro* strand exchange (>2000 base pairs of DNA are exchanged per STPα molecule) might explain how meiotic recombination can be achieved by a relatively small number of STPα molecules (≈1000 molecules per cell) with the help of ySSB.

Note. During review of this manuscript, Kolodner's group described the purification of a strand-exchange protein from mitotically growing yeast cells that also does not require any nucleotide cofactor for strand exchange (19).

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1. Orr-Weaver, T. L. & Szastak, J. W. (1985) *Microbiol. Rev.* **49,** 33–58.
2. Resnick, M. A. (1987) in *Meiosis,* ed. Moens, P. (Academic, New York), pp. 157–210.
3. Klapholz, S., Waddell, C. S. & Esposito, R. E. (1985) *Genetics* **110,** 187–216.
4. Borts, R. H., Lichten, M. & Haber, J. E. (1986) *Genetics* **113,** 551–567.
5. Resnick, M. A., Sugino, A., Nitiss, J. & Chow, T. (1984) *Mol. Cell. Biol.* **12,** 2811–2817.
6. Hotta, Y., Tabata, S., Bouchard, R. A., Pinon, R. & Stern, H. (1985) *Chromosoma* **93,** 140–151.
7. Symington, L. S., Morrison, P. T. & Kolodner, R. (1984) *Cold Spring Harbor Symp. Quant. Biol.* **49,** 805–814.
8. Cox, M. M. & Lehman, I. R. (1987) *Annu. Rev. Biochem.* **56,** 226–262.
9. Messing, J. (1983) *Methods Enzymol.* **101,** 20–78.
10. Alberts, B. M. & Herrick, G. (1971) *Methods Enzymol.* **21,** 198–217.
11. Sugino, A. & Cozzarelli, N. R. (1980) *J. Biol. Chem.* **255,** 6299–6306.
12. Sugino, A., Peebles, C. L., Kreuzer, K. N. & Cozzarelli, N. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4767–4771.
13. Davis, R. W., Simon, M. & Davidson, N. (1971) *Methods Enzymol.* **21,** 413–428.
14. Formosa, T. & Alberts, B. M. (1986) *J. Biol. Chem.* **261,** 6107–6118.
15. Cox, M. M., Soltis, D. A., Livheh, Z. & Lehman, I. R. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **33,** 803–810.
16. Radding, C. M. (1983) *Annu. Rev. Genet.* **16,** 406–437.
17. Hsieh, P., Meyn, M. S. & Camerini-Otero, R. D. (1986) *Cell* **44,** 885–894.
18. Dykstra, C. C., Nitiss, J., Resnick, M. A. & Sugino, A. (1988) in *Mechanisms and Consequences of DNA Damage Processes,* eds. Hanawalt, P. & Friedberg, E. C. (Liss, New York), in press.
19. Kolodner, R., Evans, D. H. & Morrison, P. T. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5560–5564.