Presynapsis and Synapsis of DNA Promoted by the STPα and Single-stranded DNA-binding Proteins from *Saccharomyces cerevisiae*

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We previously purified an activity from meiotic cell extracts of *Saccharomyces cerevisiae* that promotes the transfer of a strand from a duplex linear DNA molecule to complementary circular single-stranded DNA, naming it Strand Transfer Protein α (STPα) (Sugino, A., Ntiss, J., and Resnick, M. A. (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85, 3683–3687). This activity, requires no nucleotide cofactor but is stimulated more than 10-fold by the addition of yeast single-stranded DNA-binding proteins (ySSBs). In this paper, we describe the aggregation and strand transfer of double-stranded and single-stranded DNA promoted by STPα and ySSB. There is a good correlation between the aggregation induced by various DNA-binding proteins (ySSBs, DBPs and histone proteins) and the stimulation of STPα-mediated DNA strand transfer. This implies that the stimulation by ySSBs and other binding proteins is probably due to the condensation of single-stranded and double-stranded DNA substrates into coaggregates. Within these coaggregates there is a higher probability of pairing between homologous double-stranded and single-stranded DNA, favoring the initiation of strand transfer. The aggregation reaction is rapid and precedes any reactions related to DNA strand transfer. We propose that condensation into coaggregates is a presynaptic step in DNA strand transfer promoted by STPα and that pairing between homologous double- and single-stranded DNA (synapsis) occurs in these coaggregates.

Synapsis promoted by STPα and ySSBs also occurs between covalently closed double-stranded DNA and single-stranded linear DNA as well as linear double-stranded and linear single-stranded DNAs in the absence of any nucleotide cofactors.

DNA strand exchange proteins are an important category of proteins that have emerged in the last decade (1–11). They have been studied in several organisms and mammalian cells and are expected to mediate homologous DNA recombination, and are expected to mediate homologous DNA recombination, some of which may code for activities that catalyze the reactions involved in recombination (13).

Recently, we have identified and purified to homogeneity (9) an activity from yeast meiotic cells that promotes DNA pairing and strand transfer between a linear double strand and a complementary, circular single strand and have named this protein yeast strand transfer protein α (STPα). This activity does not require any nucleotide cofactor but is greatly stimulated by the addition of yeast single-stranded DNA-binding proteins. Kolodner et al. (8) have also identified, and purified from mitotically growing yeast cells, a protein that promotes an ATP-independent strand exchange reaction. The relationship between the activity from meiotic cells and that from mitotic cells is not known at this point.

Since DNA strand exchange reactions previously described in other systems require ATP (1, 2, 4–6), a major question about the ATP-independent DNA strand exchange reaction promoted by STPα and ySSB is whether it requires energy and, if so, from what source? To gain some insights into this ATP-independent reaction, we present here an investigation into the stimulatory effect of ySSBs during the DNA strand exchange reaction promoted by STPα. We find that in addition to ySSBs, any agent that generates coaggregates between ss- and dsDNA also stimulates DNA strand transfer promoted by STPα. We will refer to the product formed from the concurrent and coincident aggregation of single-stranded and double-stranded DNA as coaggregates. Examples of such agents are other yeast DNA-binding proteins, histone proteins, and spermidine. SSBs that cannot aggregate DNA also do not stimulate STPα activity. These results strongly suggest that the formation of coaggregates is a presynaptic step for ATP-independent DNA strand transfer in yeast. While STPα is not required for presynapsis, it is required for pairing between homologous double- and single-stranded DNA (synapsis). STPα also promotes ATP-independent strand transfer between a covalently closed dsDNA and linear ssDNA in the presence of ySSB. Based on these observations, we will discuss a model for the mechanism of DNA strand transfer promoted by STPα and ySSB.

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**EXPERIMENTAL PROCEDURES**

*Proteins and DNA—The purification of *S. cerevisiae* STPα and 20-kDa ySSB were described previously (9). The 14-, 26-, 35-, 40-, 42-, and 55-kDa ySSBs were isolated using a methodology described by others for purification of yeast SSBs (14, 15). Briefly, SSBs were enriched for by chromatography on single-stranded DNA cellulose, then purified by chromatography on hydroxylapatite, heparin-Sep.

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*The abbreviations used are: STPα, DNA strand transfer protein α (we have named the meiotic DNA strand transfer activity STPα in order to distinguish it from an immunologically different DNA strand transfer activity from mitotic cells); ySSB, yeast single-stranded DNA-binding protein; ds-, double-stranded; ss-, single-stranded; RF-I, Replicative Form I (supercoiled); RF-II, Replicative Form II (nicked, relaxed); RF-III, Replicative Form III (linear).
aroise, gel filtration, and Mono S. These proteins were more than 95% pure as judged by analysis of Coomassie Blue staining of SDS-polyacrylamide gels and did not contain any detectable DNase (either endo- and exonuclease) or topoisomerase activities under the conditions used for measuring the DNA strand transfer reaction. Yeast DNA-binding proteins DBP I, II, and III (16, 18) were obtained from Dr. C. Wang, Harvard University. calf thymus histone H1 and other histone proteins were purchased from Boehringer Mannheim, respectively. Yeast histone proteins were purified as described before (17). Restriction endonucleases, T4 polynucleotide kinase, bacterial alkaline phosphatase, φX174 viral single-stranded DNA, its RF-I and RF-II DNA were purchased from Bethesda Research Laboratories. Restriction endonuclease-linearized RF DNA (RF-III) of both φX174 and M13mp18 were the same as previously described (9). φX174 linear viral single-stranded DNA was prepared as follows: 250 μg of φX174 viral single-stranded circular DNA was annealed in a reaction mixture (1.25 ml) containing 20 mM Tris-HCl, pH 7.9, 10 mM MgCl₂, 100 mM NaCl, 2 mM dithiothreitol at 65 °C for 60 min with 20 μg of an oligonucleotide containing the XhoI recognition site complementary to nucleotides 154 and 175 of φX174 viral DNA (18). The reaction mixture was cooled slowly to room temperature, 500 μl of XhoI was added, and the incubation was continued as the DNA strand transfer reaction containing “P-labeled 32P-labeled φX174 RF-I11 DNA was prepared as follows. Escherichia coli C cells were grown at 37 °C in 500 ml of low phosphate media (19) to 2.5 × 10⁸ cells/ml at 37 °C. Five min after the addition of 5 MCl of [³²P]phosphate, φX174am3 phage were added at a multiplicity of infection of 2.5. After 3.5-h incubation at 37 °C, the cells were pelleted in a GSA rotor at 10,000 rpm for 10 min, resuspended in 10 ml of 0.1 M sodium borate and lysed by the addition of 10 mg of lysozyme and 1 ml of 4% EDTA and incubation at room temperature for 90 min. The lysate was sonicated with 3-s pulses until the viscosity was reduced. CsCl was added to 0.6 g/ml, and the sample was centrifuged in a Beckman VTi50 rotor for 18 h at 40,000 rpm. The opalescent band at the middle of the tube was collected and dialyzed against 0.1 M sodium borate. The DNA was extracted from the phage by phenol. The specific activity of ³²P-labeled DNA was about 1.5 × 10⁶ cpm/μg. The ³²P-labeled viral DNA was linearized in the same way as described above. ³²P-labeled φX174 viral ssDNA was prepared as published (20).

DNA Strand Transfer Reaction—The reaction mixture (0.02 ml) contained 35 mM Tris-HCl, pH 7.9, 10 mM MgCl₂, 2 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 0.2 μg of φX174 viral ssDNA, 0.4 μg of φX174 RF-III DNA (9), 2.4 μg of the 26-kDa ySSB and 20-40 ng of STPa (Fraction VI) (9). The reactions were carried out at 30 °C for the times indicated. In order to analyze the reaction products by agarose gel electrophoresis, the reactions were terminated by the addition of EDTA to 10 mM, the product was treated with phenol, precipitated with 70% ethanol, and resuspended in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA. The 22-mer oligonucleotide was synthesized with a Vega Coder 300 DNA synthesizer. ³²P-labeled φX174 viral DNA was prepared as follows. Escherichia coli C cells were grown at 37 °C in 500 ml of low phosphate media (19) to 2.5 × 10⁸ cells/ml at 37 °C. Five min after the addition of 5 MCl of [³²P]phosphate, φX174am3 phage were added at a multiplicity of infection of 2.5. After 3.5-h incubation at 37 °C, the cells were pelleted in a GSA rotor at 10,000 rpm for 10 min, resuspended in 10 ml of 0.1 M sodium borate and lysed by the addition of 10 mg of lysozyme and 1 ml of 4% EDTA and incubation at room temperature for 90 min. The lysate was sonicated with 3-s pulses until the viscosity was reduced. CsCl was added to 0.6 g/ml, and the sample was centrifuged in a Beckman VTi50 rotor for 18 h at 40,000 rpm. The opalescent band at the middle of the tube was collected and dialyzed against 0.1 M sodium borate. The DNA was extracted from the phage by phenol. The specific activity of ³²P-labeled DNA was about 1.5 × 10⁶ cpm/μg. The ³²P-labeled viral DNA was linearized in the same way as described above. ³²P-labeled φX174 viral ssDNA was prepared as published (20).

DNA Strand Transfer Reaction—The reaction mixture (0.02 ml) contained 35 mM Tris-HCl, pH 7.9, 10 mM MgCl₂, 2 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 0.2 μg of φX174 viral ssDNA, 0.4 μg of φX174 RF-III DNA (9), 2.4 μg of the 26-kDa ySSB and 20-40 ng of STPa (Fraction VI) (9). The reactions were carried out at 30 °C for the times indicated. In order to analyze the reaction products by agarose gel electrophoresis, the reactions were terminated by the addition of 1% NaDodSO₄, 10 mM EDTA, and 5% glycerol, and the samples were directly applied to a 1% agarose gel (9). Occasionally, the samples were further treated with 0.1 mg/ml Proteinase K following the addition of STPa are required as well as 26-kDa ySSB. The reaction mixture was cooled slowly to room temperature, 500 μl of XhoI was added, and the incubation was continued as the DNA strand transfer reaction containing ³²P-labeled φX174 RF-III DNA (9) and ³²P-labeled φX174 viral single-stranded circular DNA according to published procedures (21). Briefly, after the incubation, the reaction mixtures were centrifuged at room temperature for 1 min in an Eppendorf minifuge, and the radioactivity in the supernatant was measured by a liquid scintillation spectrometer.

Electron Microscopy—After reacting, the DNA samples were prepared for electron microscopy by treatment with 1% NaDodSO₄, 10 mM EDTA, 0.1 mg/ml Proteinase K for 20 min at room temperature and extraction with an equal volume of ethanol. The samples were then purified through a Bio-Gel A-0.5m column as previously described (22, 23). Formamide spreading and platinum shadowing of the DNA molecules was the same as published by Chow and Broker (24). Molecules were viewed with a Jeol 100S electron microscope and length measurements were made as published (22).

Other Methods—Other methods in this study were the same as described previously (9).

RESULTS

DNA Aggregation Is a Crucial Step for DNA Strand Transfer Catalyzed by STPa—As is observed for the RecA system (21, 25), DNA aggregation may be required for the ATP-independent DNA strand transfer promoted by STPa. We therefore determined DNA aggregation and STPa-promoted strand transfer in response to the 26-kDa ySSB. Although the 20-kDa ySSB was used previously for stimulation of STPa activity (9), we use the 26-kDa ySSB in these studies because it is the native form of the 20-kDa ySSB. We have not detected any biochemical differences between them so far. DNA aggregation was measured as the fraction of DNA that sedimented in a 1-min centrifugation (21). DNA strand transfer activity was measured at the same time using the agarose gel electrophoresis assay (9). As the concentration of 26-kDa ySSB increased, there was a sharp transition from no aggregation to complete aggregation in the presence of both ds- and ssDNA; the transition midpoint was about 120 μg/ml (Fig. 1). DNA strand transfer activity showed a similar dependence on the concentration of 26-kDa ySSB. There was a correlation between the stimulation of activity and the aggregation curve up to 120 μg/ml, with higher concentrations of 26-kDa ySSB inhibiting the DNA strand transfer reaction (Fig. 1). In the presence of ssDNA alone, at the same concentration that was used in the strand transfer assay, about 30 μg/ml ySSB aggregated 50% of the ssDNA, while approximately 90 μg/ml of ySSB was able to aggregate 50% of dsDNA (Fig. 1). Aggregation of ds- and ssDNA therefore requires the total amount of ssDNA necessary to aggregate both ss- and dsDNA alone. Unlike strand transfer, aggregation of both ds- and ssDNA did not require homologous DNA combinations: φX174 RF-III DNA + M13 viral DNA and M13 RF-III DNA + φX174 viral DNA behaved the same way as φX174 RF-III DNA + φX174 viral DNA (Fig. 1 and data not shown). For DNA strand transfer, homologous DNA combinations and the addition of STPa are required as well as 26-kDa ySSB and Mg²⁺ (Ref. 9 and Fig. 1). Under the conditions shown in Fig. 1, the addition of STPa had no effect on DNA aggregation (data not shown). We conclude that DNA aggregation
primarily a property of the 26-kDa ySSB and not of STPa. A requirement for aggregation of both ds- and ssDNA as a prerequisite for the strand transfer reaction is suggested by the experiments shown in Figs. 2 and 3. Aggregation of ds- and ssDNA by ySSB and STPa showed the same salt sensitivity as DNA strand transfer (Fig. 2) in a manner similar to that seen for the RecA protein (26). The DNA aggregation reaction in the presence of 26-kDa ySSB is rapid with the reaction complete within 5 min at 30°C and precedes the DNA strand-transfer reaction (Fig. 3). Therefore, these data are consistent with the idea that formation of coaggregates is a crucial step for DNA strand transfer catalyzed by STPa.

Aggregation and DNA Strand Transfer in the Presence of Other DNA-binding Proteins—A comparison of several other DNA-binding proteins was done in order to study the general relationship between DNA aggregation and stimulation of strand transfer promoted by STPa. The DNA-binding proteins used were yeast DBPs I, II, and III, 14-, 20-, (9), 35-, 40-, 42-, and 55-kDa ySSBs, yeast histone proteins, calf thymus histone H1 and other histone proteins, E. coli SSB, and bacteriophage T4 gene 32 protein. The results are shown in Fig. 4 and Table I. If DNA aggregation is due to counterion condensation (29), then acidic proteins are not expected to be good DNA aggregating agents. The isoelectric point for both the E. coli SSB and T4 gene 32 proteins is acidic (27), and we did not observe the formation of DNA aggregates by these proteins. For these two proteins, stimulation of the DNA strand transfer reaction catalyzed by STPa was less than 2-fold.

DNA-binding protein I (DBP I) of yeast is required for the cationation reaction catalyzed by yeast Topoisomerase II (16). This implies that DBP I also generates DNA aggregates, since DNA aggregation is required for the cationation reactions catalyzed by all known DNA topoisomerases (28). We expected that DBP I would also stimulate the strand transfer reaction promoted by STPa. Surprisingly, yeast DBP I showed very poor aggregate formation and poor stimulation of DNA strand transfer (Fig. 4D). Neither DNA aggregation nor stimulation of the DNA strand-transfer reaction could be observed in the presence of DBP III (Fig. 4F). On the other hand, DBP II was a good DNA aggregant, and the concentration giving roughly 50% aggregation of both ds- and ssDNA maximally stimulated the DNA strand transfer reaction (Fig. 4E). Among the yeast SSBs, the 14-, 26- (a native form of 20-kDa), and 35-kDa ySSBs were the best for both DNA aggregate formation and DNA strand transfer although the optimal concentration of each ySSB was different (Figs. 1 and 4). The

### Table I

| Proteins        | Molecular weight | pI  | DNA aggregation | Stimulation of STPa |
|-----------------|-----------------|-----|-----------------|---------------------|
| ySSB14          | 14,000          | >8  | Yes             | Yes                 |
| ySSB20          | 20,000          | ND  | Yes             | Yes                 |
| ySSB26          | 26,000          | ND  | Yes             | Yes                 |
| ySSB35          | 35,000          | >8  | Yes             | Yes                 |
| ySSB40          | 40,000          | 5.8 | No              | No                  |
| ySSB42          | 42,000          | 5.8 | No              | No                  |
| ySSB55          | 55,000          | 5.7 | No              | No                  |
| yDBP I          | 31,000          |     | Slightly         | Slightly            |
| yDBP II         | 14,000 and 20,000 | Yes | Yes            | Yes                 |
| yDBP III        | 22,500          |     | No              | No                  |
| Calf histone H1 |                 | Yes | Yes             | Yes                 |
| Calf other histones |            | Yes | Yes             | Yes                 |
| Yeast histones  |                 | Yes | Yes             | Yes                 |
| E. coli SSB     | 18,873          | 6.0 | No              | Slightly            |
| T4 gp32         | 33,488          | 5.0 | NO              | Slightly            |

* As measured by the centrifugation assay.

* As measured with the gel assay.

* Extremely basic. ND, not determined.

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**Fig. 2.** Salt sensitivity of DNA aggregation and strand-transfer activities by STPa and 26-kDa ySSB. The DNA aggregation and strand transfer activities were measured in the presence of 2 units of STPa and 2.4 µg of 26-kDa ySSB and the indicated concentrations of either potassium acetate or sodium chloride. ø, aggregation of 32P-labeled dsDNA; □, aggregation of 3H-labeled ssDNA; O, DNA strand-transfer activity.

**Fig. 3.** Time course of DNA aggregation and strand transfer promoted by STPa and 26-kDa ySSB. The reaction mixture contained 2.4 µg of 26-kDa ySSB, the optimal concentration for DNA strand transfer activity of STPa, 2 units of STPa, 0.4 µg of 32P-αX174 RF-III, and 0.2 µg of 3H-αX174 ssDNA in 20 µl. After incubation at 30°C for the indicated periods, DNA aggregation and strand transfer activities were analyzed. O, 32P-label in aggregates; □, 3H-label in aggregates.

**Fig. 4.** Correlation between DNA aggregation and DNA strand transfer activities in the presence of various yeast DNA-binding proteins. Aggregation of αX174 ssDNA (A), αX174 RF-III DNA (B), and αX174 RF-III DNA (C), and αX174 RF-III DNA (D) were measured in the presence of various DNA-binding proteins as described in Fig. 1. At the same time, the DNA strand transfer activity (E) was measured by gel electrophoresis as previously described (9). A, with 14-kDa ySSB; B, with 35-kDa ySSB; C, with 26-kDa ySSB (SSB1 (15)); D, with DBP I (16); E, with DBP II (16); and F, with DBP III.
remaining ySSBs neither stimulated DNA strand transfer nor formed DNA aggregates.

As shown in Fig. 5, histone H1 from calf thymus is a good aggregator and also stimulates DNA strand transfer catalyzed by STPα. However, some differences were observed between ySSB and calf histone H1 protein: the same concentration of proteins also aggregated DNA and stimulated DNA strand transfer promoted by STPα (Fig. 5), the maximum stimulation of pairing is 2-5-fold lower, and the transition midpoint from no aggregation to complete aggregation is not as sharp as for the 26-kDa ySSB. Other calf thymus histone proteins (H2A, H2B, H3, and H4) and yeast histone proteins (a mixture of all histone proteins) also aggregated DNA and stimulated DNA strand transfer promoted by STPα (Table I).

The proteins used in this study are similar in their affinity for binding to DNA but have different effects on the DNA once they are bound. In all cases, those proteins that are able to aggregate DNA stimulate STPα-promoted strand transfer. Conversely, those proteins that are incapable of aggregating DNA show little or no stimulation of STPα strand transfer.

Spermidine Can Substitute for ySSB to Stimulate DNA Strand Transfer Promoted by STPα—Spermidine is known to aggregate DNA and to stimulate the DNA catenation reaction catalyzed by DNA topoisomerases (29). If DNA strand transfer promoted by STPα requires a similar sort of DNA aggregate, then spermidine should also stimulate the strand transfer reaction. As shown in Fig. 6, spermidine not only generates DNA aggregates, but also stimulates the strand transfer reaction catalyzed by STPα. Although the maximum stimulation was 4-fold lower than that seen with the 26-kDa ySSB, spermidine was similar to the other aggregating agents in stimulating maximum strand transfer activity at a concentration that causes approximately 50% DNA aggregation. Both DNA aggregation and DNA strand transfer in the presence of spermidine were very salt sensitive, with 50% inhibition by 50 mM KCl (data not shown). This is similar to the observations of DNA aggregation and catenation of relaxed DNA by E. coli DNA gyrase (29).
DNA Synapsis by STPα—After establishing a presynaptic complex (DNA aggregation), synapsis formation (pairing of homologous DNA) could be the next step during the DNA strand exchange reaction promoted by STPα. We have analyzed the synapsis reaction by measuring the formation of stable “D-loop” structures using φX174 RF-I and its linear viral DNA by both agarose gel electrophoresis (22, 30) and electron microscopy. As shown in Fig. 7A, new DNA bands migrating much more slowly than either nicked or covalently closed, relaxed RF DNA were produced by STPα in the presence of ySSBs. Although these were evident on an ethidium bromide-stained agarose gel, they were much clearer on the autoradiograph of the dried gel (Fig. 7B). The formation of these bands required Mg²⁺, homologous DNA, and stoichiometric amounts of ySSB, but no nucleotide cofactor, in a manner identical to the conditions for the strand transfer reaction between linear dsDNA and circular ssDNA (9). To verify that these bands represent DNA synapsis products (e.g. D-loop structures), the reaction products were examined by electron microscopy. Fig. 8 shows some representative DNA molecules seen in the products. As shown in Table II, about 10% of dsDNA was an initial synapsis molecule (joint molecule) which has a single strand tail without an obvious D-loop structure and about 5% was a D-loop structure with varying sizes of paired regions and one or two tails of ssDNA (Fig. 8, B–E). Most of the remaining DNAs were the structures expected from the starting materials (supercoiled ds- and linear ssDNAs) (Fig. 8A) or large aggregates. If the DNA was not treated with Proteinase K and/or NaDodSO₄ before electron microscopy, only large aggregates were seen, as expected from the action of the 20- to 26-kDa ySSB (data not shown).

Strand Transfer between Linear ds- and Linear ssDNAs—As shown in Fig. 9, left, STPα and ySSB promote strand transfer between φX174 RF-III DNA and φX174 linearized viral ssDNA. The new DNA bands can be seen easily on an agarose gel. Autoradiography of the dried gel confirms that these new bands are in fact joint molecules between RF-III and linear ssDNA (Fig. 9, right). The joint molecule formed from RF-III and linear ssDNA migrates similarly to the joint molecule formed from RF-III and circular ssDNA because of their equivalent mass. Conversion of the RF-III DNA to joint molecules was almost complete. Like the previously described reactions, this strand transfer required homologous DNAs, Mg²⁺, and ySSB (data not shown).

DISCUSSION

DNA strand transfer is believed to be one of the steps involved in homologous DNA recombination (1). Thus, proteins which promote DNA strand transfer in vitro may be important for homologous recombination in both prokaryotic and eucaryotic systems. In some cases, this has been proven genetically (1). In these cases, the proteins all require a nucleotide cofactor for the reaction and are DNA-dependent NTPases. Recently, however, strand transfer proteins have been isolated from human (3), yeast (8, 9), and Drosophila melanogaster cells (10, 11) which differ from the previously described strand transfer proteins in not requiring any nucleotide cofactors. STPα from S. cerevisiae meiotic cells contained no detectable endo- or exonuclease or ATPase activities and promoted extensive strand transfer in the presence of ySSB (9). We have suggested that strand transfer does not require energy from ATP hydrolysis and that the mechanism is different from that of the previously described reactions.

![Fig. 8. Electron microscopic analysis of the synapsis products formed by STPα and 26-kDa ySSB. The reaction identical to Lane 8 in Fig. 7 was terminated by addition of 10 mM EDTA and 1% SDS and treated with 100 µg/ml Proteinase K at room temperature for 15 min. The sample was extracted with an equal volume of phenol, purified by gel filtration, and analyzed by electron microscopy as previously described (22–24). A, φX174 linear viral ssDNA and RF-I DNA. B–E, D-loop structures. The size of the D-loops observed ranged between 100 and 2500 base pairs. A bar in E equals 0.5 µm. Open triangles in B–E show the region where a single-stranded DNA might be tangled around either a single-stranded or double-stranded DNA. The right-hand side of each electron micrograph shows an interpretation of the picture. Thick and thin lines represent double-stranded and single-stranded DNA, respectively.](image-url)

### Table II

| Reaction | Total molecules counted | RF-I | RF-II | Joints | D-loops | Aggregates |
|----------|------------------------|------|-------|--------|---------|------------|
| 1) RF-I + linear ssDNA | 100 | 96 | 4 | | | |
| 2) 1) + STPα + 26 kDa SSB | 454 | 70.2 | 5 | 10 | 4.8 | 9 |

* Only double-stranded DNA molecules were counted.

* Joint molecules were characterized as ds- and ssDNA interactions without an obvious D-loop structure.
The possibility that STPα has an unidentified high energy source has not yet been ruled out. This is, however, unlikely because each STPα molecule promotes the transfer of more than 2000 base pairs (9) and any inherent energy should be quickly consumed at the beginning of the reaction.

In order to gain a further understanding of this ATP-independent strand transfer reaction, we have studied the STPα-promoted reaction in more detail. We have found that yeast DNA-binding proteins or spermidine play a crucial role for strand transfer promoted by STPα. Among the ySSBs and DBPs tested in this paper, the 14-, 20-, 26-, and 35-kDa ySSBs and DBP II stimulated the strand transfer reaction significantly (more than 20-fold). These proteins also promoted the formation of DNA aggregates. Histone H1 and spermidine, which are known to be good DNA aggregants (29), also stimulated DNA strand transfer to almost the same extent as ySSBs. The correlation between aggregation of both ds- and ssDNA and stimulation of DNA strand transfer implies that coaggregates of ss- and dsDNA are readily accessible to STPα and that within these coaggregates the search for homology is facilitated by the high local concentrations of DNA. Optimal concentrations of various DNA-binding proteins and spermidine for the strand transfer reaction catalyzed by STPα is approximately the amount that generates 50% substrate DNA aggregation. Concentrations resulting in higher than 50% aggregation inhibited DNA strand transfer. This is similar to the observations on the catenation reaction catalyzed by DNA topoisomerases (29). What significance does the aggregation of DNA have to homologous pairing in vivo? In all of the in vitro DNA pairing reactions reported thus far (9 and in this report), the substrate DNA concentrations are on the order of micromolar, far less than the millimolar DNA concentrations expected to be present in the yeast nucleus. Therefore, one interpretation of why DNA aggregation facilitates DNA strand transfer promoted by STPα in vitro is that a high DNA concentration is necessary to support efficient homologous pairing and that aggregation promotes these pairing conditions. Since the effective DNA concentration in vivo is already very high, this interpretation implies that DNA aggregation and agents that induce aggregation may not have any specific relevance to in vivo pairing. Alternatively, the formation of an aggregated complex of DNA and proteins, such as nucleosome structure, could be integral to the location of homologous sequences in vivo and may help account for the remarkable efficiency of homologous recombination in yeast.

Based on the data so far (9 and this study), a model for the mechanism of the DNA strand transfer reaction promoted by STPα and ySSB is presented in Fig. 10. The first stage of the reaction is the binding of ySSB to DNA and subsequent presynaptic complex formation (DNA aggregation). Here, we predict that 20- or 26-kDa ySSB binds to ssDNA first, due to preferential binding to ssDNA over dsDNA (13). This stage does not require STPα or homologous DNAs. The second stage involves searching for homology and formation of synaptic complexes (D-loop structures) which absolutely require homologous DNAs and STPα. It is not yet known whether paranemic joints are made before the formation of plectonemic joints (stable D-loop structures) (1). Technically it has been impossible to detect paranemic joints by filter binding experiments and/or electron microscopy, since ySSB alone aggregates DNAs so efficiently at the early stages of the reaction. The last stage presented here is branch migration to form a final product, an RF II-like molecule and displaced linear ssDNA. More complicated products are commonly observed in the reaction suggesting that the displaced ssDNA might be a preferred substrate for further invasion of dsDNA, leading to a network of partial strand transfer products (8). This inefficient completion of strand transfer may be related to the absence of a nucleotide cofactor requirement for the reaction or to the lack of some other factor(s) required for complete DNA strand transfer. The T4 ussX protein, like STPα, also produces complex DNA strand transfer reaction products under certain conditions (32, 33). The T4 ussX protein produces only a small amount of RF II DNA and, in the presence of T4 gene 32 protein, the displaced linear ssDNA further invades dsDNA to form complicated network-like structures during strand transfer (32, 33). Unlike STPα,

![Fig. 9. DNA strand transfer between linear ds-DNA and either circular or linear ssDNA. DNA strand transfer was carried out as in Fig. 1 except that 32P-labeled ϕX174 viral (either linear or circular) ssDNA was used. After incubation at 30 °C for 30 min, the products were analyzed by agarose gel electrophoresis, visualized by ethidium bromide staining (left-hand figure), followed by autoradiography (right-hand figure) as in Fig. 7. A, ϕX174 RF-III plus ϕX174 viral circular ssDNA. B, ϕX174 RF-III plus ϕX174 viral linear ssDNA. C, A plus 2.4 μg of 26-kDa ySSB and 2 units of STPα. D, the same as C except for 4 units of STPα. E, the same as C except for 8 units of STPα. F-H, the same as C-E, respectively, except the ϕX174 viral DNA was linearized. For lanes a-h, each lower-case letter matches the capital letter in the stained gel.](image-url)

![Fig. 10. Proposed model for DNA strand transfer promoted by STPα in the presence of ySSB. A detailed explanation is given in the text. Small circles (○) and triangles (▲) represent ssDNA-binding protein and STPα molecules, respectively. Generation of a free single-stranded linear molecule has not been detected after the reaction (9). The complete DNA strand transfer reaction might require additional factors which have not been identified.](image-url)
however, T4 uvsX protein efficiently generates a typical RF II DNA and a free displaced linear ssDNA in the presence of E. coli SSB instead of gene 32 protein (32).

By comparing the reaction of STPα with ySSB with that promoted by E. coli RecA protein (and E. coli SSB), some major differences are evident besides the lack of a nucleotide cofactor requirement. One of these is the role of ySSB during strand transfer. In the case of the reactions promoted by RecA, the current model has RecA molecules binding first to ssDNA, forming a special conformation of the DNA. Subsequently, E. coli SSB displaces RecA protein coated on ssDNA before the reaction can proceed. Thus, for RecA it is very important when and in which order each of the proteins are added (1). The DNA strand transfer catalyzed by STPα and ySSB does not require any specific order of addition. Since a few molecules of STPα promote extensive DNA strand transfer, it is likely that ssDNA first interacts with ySSB and subsequently STPα displaces ySSB molecules on ssDNA during the reaction. ySSB itself generates DNA aggregation which could be a crucial step for the initiation of the strand transfer reaction, while RecA protein alone generates DNA aggregation. We speculate that ySSB might share a part of the roles played by E. coli RecA protein during DNA strand transfer. Another difference is that the levels of RecA protein increase during recombination while the amount of STPα protein remains low, and constant, during meiosis although its specific activity increases (data not shown). The mechanisms for increasing the levels of strand transfer activity when recombination is induced appear to differ in yeast and E. coli.

Our current work involves the comparison of the mitotic and meiotic STP activities, both biochemically and genetically. The preliminary evidence indicates that the mitotic activity acts in a very similar manner to the meiotic activity but with a lower efficiency. This correlates well with genetic observations of recombination frequencies during mitotic growth versus meiosis (13).

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