Purification and Characterization of a Protein from *Saccharomyces cerevisiae* That Binds Tightly to Single-Stranded DNA and Stimulates a Cognate Strand Exchange Protein†

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ABSTRACT: A single-stranded DNA binding protein (yeast SSB protein) was purified to near-homogeneity from mitotic *Saccharomyces cerevisiae* cells. The M_r 34000 protein specifically eluted at high salt (~1200 mM NaCl) during chromatography on a single-stranded DNA–cellulose column. The protein formed stable complexes with single-stranded DNA in an apparent cooperative fashion. As judged from titration and competition experiments, the affinity of the protein was much higher for single-stranded DNA than for double-stranded DNA or single-stranded RNA. The SSB protein also was found to stimulate the strand exchange reaction between linear M13mp19 RF DNA and circular M13mp19 viral DNA as catalyzed by a yeast strand exchange protein previously purified in this laboratory [Kolodner, R., Evans, D. H., & Morrison, P. T. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5660–5664]. Titration experiments showed maximum stimulation of joint molecule formation at a stoichiometry of about 1 M_r 34000 monomer yeast SSB per 18 nucleotides of single-stranded DNA. Kinetic experiments demonstrated at least an 18-fold increase in the rate of strand exchange due to the presence of the SSB in reactions where the amount of strand exchange protein was limiting. The yeast SSB protein stimulated the *Escherichia coli* RecA protein in the strand exchange reaction involving linear M13mp19 RF DNA and circular M13mp19 viral DNA as efficiently as *E. coli* SSB. However, the *E. coli* SSB protein did not substitute for the yeast SSB protein in reactions with the yeast strand exchange protein. This suggests that the stimulation of the yeast strand exchange protein by the yeast SSB may involve specific protein/protein interactions.

The formation of hybrid DNA is one of the central processes of genetic recombination. Most of our understanding of hybrid DNA formation derives from genetic and biochemical analysis of the *Escherichia coli* RecA protein, a protein which is involved not only in recombination but also in the repair of DNA damage and the regulation of the SOS response [for a review, see Clark (1973) and Smith (1988)]. Although RecA catalyzes the formation of hybrid DNA from a variety of different DNA substrates [reviewed in Radding (1982) and Cox and Lehman (1987)], one of the most extensively analyzed model reactions is the formation of joint molecules from linear duplex DNA and circular single-stranded DNA (ssDNA).[1] In this reaction, RecA binds stoichiometrically to the ssDNA to form an activated intermediate which then invades the dsDNA (McEntee et al., 1981; Stasiak et al., 1984; Tsang et al., 1985). The formation of stable joint molecules requires a free homologous 3′ end on the linear duplex, and the branch migration proceeds in a polar fashion in the 3′ to 5′ direction (Kahn et al., 1981; Cox & Lehman, 1981b).

It was discovered that the *E. coli* SSB protein stimulated the strand transfer activity of RecA (McEntee et al., 1980; Shibata et al., 1980; Cox & Lehman, 1981a). Under conditions where the RecA concentration is limiting, strand transfer becomes dependent on the presence of SSB (Cox et al., 1983). Detailed studies on the mechanism of stimulation by SSB argued against models in which the stimulation was mediated by specific protein/protein interaction. Rather, the SSB seems to be required to melt out potential secondary structures in the ssDNA which apparently impair the strand exchange activity of RecA (Muniyappa et al., 1984). In support of this mechanism, other SSBs, such as phage T4gp32 or phage λ β-protein, were found to substitute for the *E. coli* SSB in the stimulation of the RecA protein (Shibata et al., 1980; Muniyappa et al., 1984; Egner et al., 1987; Chow et al., 1988). Recently, Radding and co-workers proposed a new model in which SSB was hypothesized to stimulate RecA by suppressing reinitiation events after initial hybrid DNA formation (Chow et al., 1988). This view is compatible with an earlier observation made by Pugh and Cox (1987), who found that in the presence of SSB the RecA protein remains stably associated with the heteroduplex DNA after strand exchange, thereby suppressing reinitiation.

A similar result has been obtained in studies with the T4 UvsX protein, which is also stimulated by T4gp32, a phage T4 SSB (Formosa & Alberts, 1986). Analysis of this interaction has concluded that the stimulation of the UvsX protein occurs at the initiation stage of the reaction and does not extend to the branch migration phase of the reaction, because the presence of T4gp32 had little effect on the rate of branch migration (Kodadek et al., 1988).

In order to gain insight into the mechanism of hybrid DNA formation in a eukaryotic system, this laboratory has described the purification and characterization of a strand exchange protein from mitotic yeast cells (Kolodner et al., 1987). The

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[1] Abbreviations: SSB, single-stranded DNA binding protein; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; ssRNA, single-stranded RNA.

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active polypeptide is an M_r 132000 species that has many features in common with the *E. coli* RecA protein including the basic mechanism of action during strand exchange, polarity of branch migration, DNA binding properties, and the ability to renature complementary single-stranded DNA (Kolodner et al., 1987; Heyer et al., 1988). The only striking difference identified so far between the two proteins seems to be the absence of an ATP requirement for the yeast protein.

In analogy to studies with RecA and UvsX, it was of interest to determine what role, if any, yeast SSBs might have on the strand transfer reaction catalyzed by the yeast strand exchange protein. Several reports about ssDNA binding proteins from yeast described the purification of a variety of proteins that were originally defined as yeast SSBs (Chang et al., 1975; LaBonne & Dumas, 1983; Jong et al., 1985). Several of these proteins, however, are more likely to be RNA binding proteins than functional analogues of the *E. coli* or T4 SSB. Furthermore, the yeast SSBs were found to bind much less tightly to ssDNA during ssDNA–cellulose chromatography than the prokaryotic SSBs. The relation of these yeast SSBs to recombination has not been studied in detail.

In our effort to identify proteins that are potentially involved in genetic recombination in the yeast *Saccharomyces cerevisiae*, we set out to identify yeast proteins that stimulate the strand exchange reaction catalyzed by the yeast strand exchange protein in vitro under conditions where strand exchange protein was limiting. This approach is similar to the method used to demonstrate a requirement for *E. coli* Fis protein in the bacteriophage λ excision reaction (Thompson et al., 1987). We have identified a yeast protein that specifically elutes at high NaCl concentrations during ssDNA–cellulose chromatography. The purified protein has properties that are consistent with being a ssDNA binding protein, in that it has low affinity to dsDNA and no measurable affinity for ssRNA. This putative yeast SSB was found to stimulate the strand exchange reaction catalyzed by the yeast strand exchange protein and also stimulated the *E. coli* RecA protein.

Materials and Methods

Strains. *S. cerevisiae* strain BJ926 (*a/α,trp1/TRP1, HIS1/his1, prc1-126/prc1-126, pep4-3/pep4-3, prb1-1122/prb1-1122, can1/can1*) was from Dr. D. Hinkle (University of Rochester, Rochester, NY). *E. coli* strain AB259 (HfrH, *thi-1, rel-1*) and bacteriophage M13mp19 were from laboratory stocks. *E. coli* strain RLM727 which is *E. coli* HfrH/pRLM55 (LeBowitz, 1985) was from Dr. Roger McMacken (Johns Hopkins School of Public Health, Baltimore, MD).

Enzymes and Chemicals. Restriction endonucleases were obtained from New England Biolabs (Beverly, MA) and used as suggested by the manufacturer. Creatine phosphokinase (type I), creatine phosphate, and calf thymus DNA (type I) were from Sigma (St. Louis, MO). RNA (0.24–9.5-kb RNA ladder) was purchased from Bethesda Research Laboratories (Gaithersburg, MD). Double-stranded and single-stranded DNA–cellulose was prepared essentially as described by Alberts and Herrick (1971), except that in the preparation for ssDNA–cellulose the solution with the denatured DNA was adjusted to neutral pH with 12 M HCl before it was mixed with the cellulose powder. Typically, ~1 mg of DNA, ds or ss, was bound to 1 mL of packed cellulose.

Nucleic Acids. To purify M13mp19 viral DNA, M13mp19 phage were obtained by standard methods and further purified by centrifugation in CsCl density gradients (Kolodner et al., 1987). M13mp19 viral DNA was extracted from the purified phage particles, and M13mp19 RFI DNA was purified from infected cells as previously described (Kolodner et al., 1987). ^{3}H-Labeled M13 DNA was obtained by growing the cells in 2 L of Fraser's medium (Fraser & Jerrel, 1953) supplemented with 0.01% thiamin to a density of OD_{590} = 0.15. Then 5 mCi of [^{3}H]thymidine (20 Ci/mmol; NEN, Boston, MA) and adenosine and deoxyadenosine, both to a final concentration of 1 mM, were added. The cells were grown until the culture reached an OD_{590} = 0.7, and then phage were added at a multiplicity of infection of 10. The preparation of ss- and dsDNA was then carried out as described above. DNA concentrations are expressed as moles of nucleotides using ϵ_{260} values of 6800 and 8500 for dsDNA and ssDNA, respectively.

Protein Preparations. (*A*) *S. cerevisiae Strand Exchange Protein.* The yeast strand exchange activity was purified through fraction V as described in Kolodner et al. (1987) and was >90% pure as judged by NaDodSO$_4$/PAGE.

(*B*) *S. cerevisiae ssDNA Binding Protein.* The growth and processing of the yeast were performed exactly as described (Kolodner et al., 1987). All subsequent operations were performed at 4 °C unless otherwise specified. In a typical purification, to 800 mL of cell suspension (=400 g) were added 1000 mL of buffer W [50 mM Tris-HCl (pH 7.5), 10% (w/v) sucrose, and 1 mM EDTA], 180 mL of 4 M KCl, 18 mL of 0.5 M spermidine, 3.6 mL of 0.5 M EDTA (pH 8.0), 1.8 mL of 2-mercaptoethanol, and 734 mg of Zymolyase T-100 (Seikagaku Kogyo Co. Ltd., Tokyo, Japan). After 2.5 h on ice, 18.2 mL of 10% (wt/v) Brij 58, 1.82 mL of 0.1 M phenylmethanesulfonyl fluoride (dissolved in 95% ethanol), and 24.6 mL of 4 M KCl (final concentration 0.4 M) were added. After 20 min on ice, the suspension was centrifuged for 25 min at 40000 rpm in a Beckman 45Ti rotor, and the supernatants were pooled to give 1930 mL of an 18.2 mg/mL solution of protein (fraction I). To fraction I was added with stirring on ice 753 g of ammonium sulfate over a 30-min period. After stirring for an additional 30 min, the precipitate was harvested by centrifugation for 30 min at 9000 rpm in a Sorvall GS3 rotor. The precipitate was resuspended to 800 mL with buffer A [20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 10% (w/v) glycerol, 10 mM 2-mercaptoethanol, and 0.1 mM phenylmethanesulfonyl fluoride], and the solution was dialyzed for 2 h against 8 L of buffer A. This yielded 850 mL of a 26.0 mg/mL protein solution with a conductivity equivalent to buffer A containing 150 mM NaCl (fraction II). Fraction II was applied at 150 mL/h to a 12.6 cm^2 × 17.5 cm column of ssDNA–cellulose equilibrated with buffer A containing 150 mM NaCl. After the column was washed with 370 mL of buffer A containing 150 mM NaCl, the protein was eluted from the column with 825 mL of a linear gradient from 150 mM NaCl to 900 mM NaCl in buffer A. After the gradient was completed and the eluate reached a conductivity equivalent to buffer A containing 800 mM NaCl, the ssDNA binding protein was eluted with 440 mL of buffer A containing 2000 mM NaCl to yield fraction III. Fraction III was dialyzed against buffer A 2 times for 2 h each to yield 210 mL of a 0.018 mg/mL solution with a conductivity equivalent to buffer A containing 70 mM NaCl. Fraction III was applied at 7 mL/h to a 0.64 cm^2 × 1.5 cm column of dsDNA–cellulose equilibrated with buffer A containing 70 mM NaCl. The column was then washed with 3 mL of buffer A containing 70 mM NaCl. The flowthrough and the wash were pooled to yield 210 mL of a 0.010 mg/mL protein solution (fraction IV). Fraction IV was applied at 10 mL/h to a 0.64 cm^2 × 1.5 cm column of ssDNA–cellulose equilibrated with buffer A containing 70 mM NaCl. The column was washed with 3 mL of buffer A containing 70 mM NaCl, and the protein

was eluted with 5 mL of buffer A containing 2000 mM NaCl. The protein-containing fractions were identified by NaDodSO$_4$/PAGE, pooled, and dialyzed overnight against 1 L of buffer A containing 60% (w/v) glycerol and 100 mM NaCl to yield 0.32 mL of fraction V (0.81 mg/mL). Fraction V was used for all experiments described below.

(*C*) *Escherichia coli RecA Protein.* RecA protein was purified essentially as described (Griffith & Shores, 1985) except that the final PBE 94 column was eluted with a salt gradient instead of by step elution. The final preparation was >98% pure as demonstrated by NaDodSO$_4$/PAGE.

(*D*) *Escherichia coli ssDNA Binding Protein. E. coli* SSB was purified from *E. coli* strain RLM727 through the ammonium sulfate fractionation step exactly as described (LeBowitz, 1985). The SSB was further purified by chromatography on ssDNA–cellulose and PBE 94 essentially as described (Williams et al., 1984). The final preparation was >98% pure as demonstrated by NaDodSO$_4$/PAGE.

Protein fractions were diluted in buffer containing 10 mM Tris-HCl (pH 7.5), 10 mM 2-mercaptoethanol, and 0.5 mg/mL bovine serum albumin. Protein concentrations were determined by the method of Lowry et al. (1951), and protein samples were analyzed by NaDodSO$_4$/PAGE (Mansernigi et al., 1977).

Assays for Strand Exchange. (*A*) *Yeast Strand Exchange Protein.* Assays were carried out in 30-μL reactions containing 33 mM Tris-HCl (pH 7.5), 13 mM $MgCl_2$, 1.8 mM dithiothreitol, 1.3 mM ATP, 3 mM creatine phosphate, 88 μg/mL bovine serum albumin, 10 units/mL creatine phosphokinase, 0.6 nmol of *Eco*RI-cleaved linear duplex M13mp19 DNA, and 0.3 nmol of circular viral M13mp19 DNA. All strand exchange reactions were incubated at 30 °C for 20 min unless otherwise indicated. In reactions with ssDNA binding proteins, the yeast strand exchange protein was added first, and the reaction mixture was kept on ice for 1 min before the respective SSB was added. After the specified incubation period, 0.5 M EDTA (pH 8.0), proteinase K (20 mg/mL) (Beckman Instruments, Palo Alto, CA), and 10% NaDodSO$_4$ were added to 50 mM, 600 mg/mL, and 0.1%, respectively, and the reactions were incubated at 37 °C for 10 min. Then $^1/_9$th volume of a solution containing 0.25% bromophenol blue, 0.25% xylene cyanol FF, 120 mM EDTA (pH 8.0), and 15% (w/v) Ficoll was added, and each sample was analyzed by electrophoresis through a 1% agarose slab gel run in buffer containing 40 mM Tris–acetate, pH 7.9, 1 mM EDTA, and 0.5 μg/mL ethidium bromide. The gels were photographed on Polaroid type 665 positive/negative film, and the negatives were quantitated by using an LKB Ultroscan laser densitometer.

(*B*) *Escherichia coli RecA.* The protocol developed by Christiansen and Griffith (1986) was generally followed. Assays were carried out in 30 μL containing 20 mM Tris-HCl (pH 7.5), 12 mM $MgCl_2$, 0.1 mM EDTA, 3 mM ATP, 15 mM NaCl, 20 mM creatine phosphate, and 4 μg/mL creatine phosphokinase. RecA protein was preincubated in the reaction mix for 10 min at 37 °C, and then 0.3 nmol of circular viral M13mp19 was added. The SSB proteins were added 2 min later; 300 ng of *E. coli* SSB or 540 ng of yeast SSB was added; in both cases, these amounts equaled 1 SSB monomer per 20 nucleotides of ssDNA. After 10-min incubation at 37 °C, 0.6 nmol of *Eco*RI-cleaved linear duplex M13mp19 DNA was added, and the incubation was continued for an additional 20 min. Subsequent proteinase K digestion, sample preparation, and gel electrophoresis were performed as described for the yeast strand exchange protein.

(*C*) *DNA Binding Assays.* Thirty-microliter reactions were carried out using strand exchange assay conditions with either 0.3 nmol of single-stranded M13mp19 viral [^{3}H]DNA (3900 cpm/nmol) or 0.3 nmol of double-stranded M13mp19 RFI [^{3}H]DNA (37 000 cpm/nmol). Incubations were for 2 min at 30 °C. After incubation, the samples were diluted 33-fold with 500 mL of ice-cold wash buffer [33 mM Tris-HCl (pH 7.5), 13 mM $MgCl_2$, and 1.8 mM dithiothreitol] and filtered through KOH-treated nitrocellulose filters (BA85, 0.45 μm; Schleicher & Schuell, Keene, NH) (McEntee et al., 1980). The filters were washed with 1 mL of wash buffer, dried for 10 min under an infrared heating lamp, and counted in 5 mL of Betafluor (National Diagnostics, Somerville, NJ) in a Beckman LS 7000 scintillation counter to determine the bound radioactivity.

Results

Identification and Purification of a ssDNA Binding Protein. We have identified and purified an M_r 34 000 polypeptide that eluted at high concentrations of NaCl during ssDNA–cellulose chromatography. Figure 1 shows the protein pattern of the elution profile of a ssDNA–cellulose column loaded with an ammonium sulfate fraction (fraction II, see Materials and Methods) of a crude extract (fraction I) from mitotic *S. cerevisiae* cells. The bound proteins were eluted with a gradient from 70 to 1500 mM NaCl, followed by a wash with 2000 mM NaCl. Although several proteins eluted from the column at high salt, an M_r 34 000 polypeptide was the only major protein specifically eluting at high NaCl concentration (midpoint ~1200 mM). This protein was purified by a modified protocol involving only differential dsDNA–cellulose and ssDNA–cellulose chromatography (see Materials and Methods) and yielded a preparation that was estimated to be >90% pure. Fraction V contained no significant dsDNA or ssDNA exonuclease or endonuclease activity, ATPase activity, or strand exchange activity. The purification of the M_r 34 000 polypeptide is summarized in Figure 2, where an electrophoretic analysis of each protein fraction is presented. On the basis of the purification criterium, this protein was hypothesized to be a yeast SSB and will be referred to by this name throughout this paper. It is difficult to estimate how much of this protein is present in the cell, because it could not be detected until after the first chromatography step. However, estimating the losses during purification at 50% and assuming that all molecules of yeast SSB present in the cell were released and present in the crude extract, we calculate a minimum of 3500 molecules of the M_r 34 000 polypeptide per mitotic cell.

It is noteworthy that essentially all of the proteins in fraction IV except the M_r 34 000 yeast SSB did not bind to the second ssDNA–cellulose column that was used to concentrate the protein (see Figure 2). This probably reflects that the nature of the retention of these proteins on ssDNA–cellulose was not the protein/ssDNA interaction.

Nucleic Acid Binding Properties of the Yeast SSB. The nucleic acid binding properties of the yeast SSB were studied by measuring the retention of stable protein/DNA complexes on nitrocellulose filters. Figure 3A shows protein titration curves which measure the complex formation between yeast SSB and ssDNA or dsDNA. The protein had a high affinity for ssDNA [Figure 3A (○)]. The sigmoidal shape of the curve suggested a certain degree of cooperativity in the binding to ssDNA; however, this point will require further analysis to substantiate this notion. The amount of protein required for half-maximal complex formation (as calculated from Figure 3A) was about 1 M_r 34 000 monomer per 2000 nucleotides of ssDNA. This is considerably less than using the yeast strand

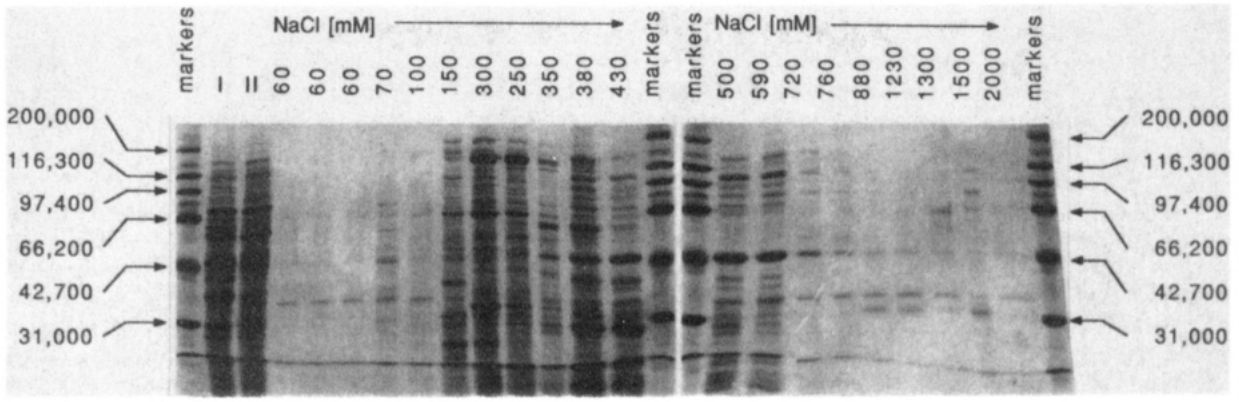

FIGURE 1: Protein elution profile of an ssDNA–cellulose column. A total of 6800 mg of fraction II (Materials and Methods) was loaded on a ssDNA–cellulose column (12.6 cm^2 × 17.5 cm) equilibrated with bufferA containing 70 mM NaCl. The column was washed with 330 mL of buffer A containing 70 mM NaCl, and the protein was eluted with a 1000-mL linear salt gradient (70–1500 mM NaCl in buffer A). After completion of the gradient, the column was washed with 220 mL of buffer A containing 2000 mM NaCl. Column fractions eluting at NaCl concentrations higher than 720 mM were desalted and concentrated 2-fold by dialysis overnight against buffer A containing 60% (w/v) glycerol and 100 mM NaCl. The protein fractions were analyzed by a $NaDodSO_4$/PAGE 16% gel. Lanes: I, 10 μg of fraction I; II, 10 μg of fraction II; 60 mM NaCl through 350 mM NaCl, 20 μL of the original fraction from the ssDNA–cellulose column; 380 mM NaCl through 590 mM NaCl, 150 μL of the original fraction; 720 mM NaCl through 2000 mM NaCl, 1400 μL of the original fraction, except for 1500 mM NaCl, where accidently more protein was loaded. All protein samples were concentrated by precipitation with trichloroacetic acid prior to electrophoresis. The markers used were myosin (M_r 200 000), β-galactosidase (M_r 116 250), phosphorylase B (M_r 97 400), bovine serum albumin (M_r 66 200), ovalbumin (M_r 42 699), and carbonic anhydrase (M_r 31 000).

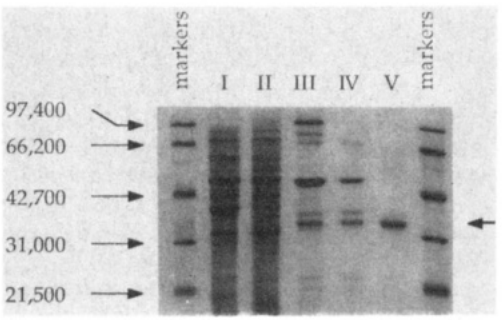

FIGURE 2: $NaDodSO_4$/PAGE analysis of purified protein fractions. The indicated lanes contained (I) 14 μg of fraction I, (II) 12 μg of fraction II, (III) 5 μg of fraction III, (IV) 4 μg of fraction IV, or (V) 0.5 μg of fraction V. Electrophoresis was on a 15% gel. The arrow indicates the position of the M_r 34 000 polypeptide. The markers used were phosphorylase B (M_r 97 400), bovine serum albumin (M_r 66 200), ovalbumin (M_r 42 699), carbonic anhydrase (M_r 31 000), and soybean trypsin inhibitor (M_r 21 500).

exchange protein which required about 1 M_r 132 000 monomer per 550 nucleotides of ssDNA for half-maximal complex formation (Heyer et al., 1988). In this assay, the yeast SSB had a similar affinity to ssDNA as the *E. coli* SSB protein as judged from the stoichiometry at half-maximal protein/DNA complex formation (data not shown). As expected from the elution profile of the yeast SSB in ssDNA–cellulose chromatography, the protein/ssDNA complexes were found to be stable in high NaCl concentrations; at a concentration of 750 mM NaCl, 80% of the protein/DNA complexes were still present.

The yeast SSB had a much lower affinity to dsDNA [Figure 3A (●)] than for ssDNA, again very similar to the *E. coli* SSB protein (data not shown). No further increase in complex formation above 40% of the dsDNA bound was measured in experiments using up to 800 ng of yeast SSB per 0.3 nmol of dsDNA (data not shown).

The relative binding affinity of the yeast SSB to different nucleic acids was determined more precisely in competition experiments (Figure 3B). Binding was assayed in reactions containing a constant amount of single-stranded M13mp19 viral [^{3}H]DNA and varying amounts of nonradioactive ssDNA (M13mp19 viral DNA) or dsDNA (M13mp19 RF DNA), or

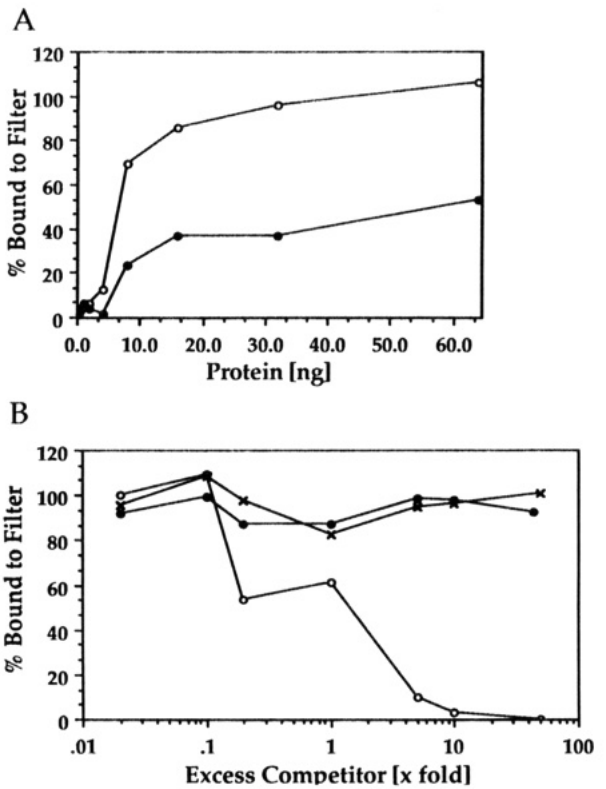

FIGURE 3: Nucleic acid binding properties of yeast SSB. (A) Titration of binding to ssDNA and dsDNA by yeast SSB. Reactions were performed as described under Materials and Methods. Each reaction contained the indicated amount of yeast SSB (fraction V) and either ssDNA (○) or dsDNA (●). (B) ssDNA binding competition experiment with yeast SSB. Reactions were performed as described under Materials and Methods. Each reaction contained 8 ng of yeast SSB (fraction V), 0.3 nmol of ss M13mp19 viral [^{3}H]DNA, and the indicated amount of unlabeled competitor DNA [(○) circular viral M13mp19 ssDNA; (●) circular replicative form M13mp19 dsDNA; (×) ssRNA]. 100% was defined as the percent radioactivity bound to the filter in the absence of any competitor, which was 82.0–84.4% of the total [^{3}H]ssDNA in this experiment.

nonradioactive ssRNA (0.24–9.5-kb RNA ladder). The amount of yeast SSB used per assay (8 ng) was slightly less than that required for maximal binding (Figure 3A) to ensure

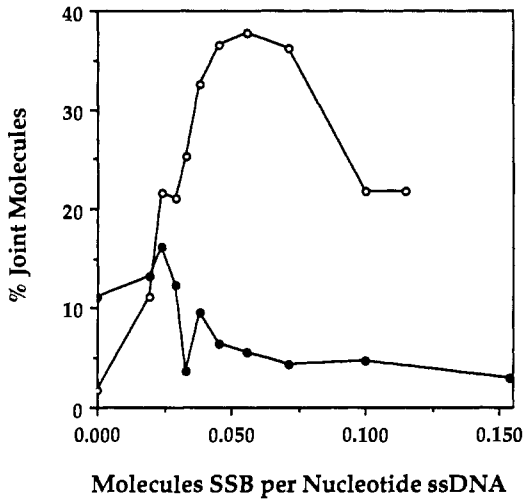

FIGURE 4: Stimulation of the yeast strand exchange protein by the yeast SSB. Strand exchange reactions were performed as described under Materials and Methods. Each reaction contained 0.84 μg of strand exchange protein (fraction V) and the indicated amount of yeast SSB (○) or *E. coli* SSB (●). To calculate the molecules of SSB per nucleotide of ssDNA, we assumed for the yeast SSB a purity of 95% of an M_r 34 000 polypeptide and for the *E. coli* SSB a purity of 95% of an M_r 18 500 polypeptide.

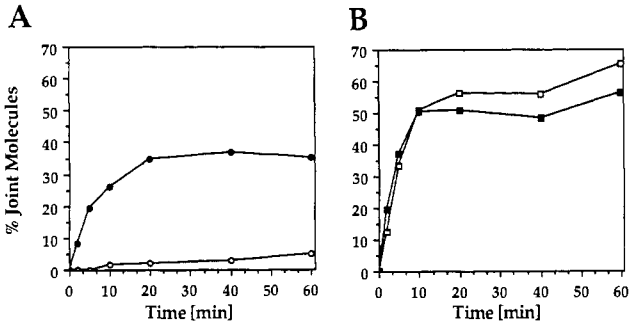

FIGURE 5: Kinetics of strand exchange stimulation by the yeast SSB. (A) The kinetics of strand exchange in the presence of limiting amounts of strand exchange protein. Strand exchange reactions (8× reactions containing 240 μL) were performed as described under Materials and Methods. Each reaction contained 8 μg of yeast strand exchange protein (fraction V), 2.4 nmol of M13mp19 viral DNA, and 4.8 nmol of linear M13mp19 RF DNA in the absence (○) or presence of yeast SSB (350 ng of fraction V) (●). Thirty-microliter aliquots were removed from the reaction at the indicated times and immediately incubated with $NaDodSO_4$/EDTA/proteinase K, as described under Materials and Methods, to terminate the reaction. The reactions were held on ice until all samples were ready for analysis by gel electrophoresis. (B) Kinetics with saturating amounts of strand exchange protein. Reactions were performed exactly as described for Figure 5A, except here reactions contained 2 μg of strand exchange protein (fraction V) per 0.3 nmol of ssDNA. Reactions in the absence (□) or presence (■) of yeast SBB.

that all protein was present as protein/DNA complexes so that competition could be detected. Figure 3B shows that nonradioactive ssDNA exhibited competition, as theoretically expected. Both dsDNA and ssRNA failed to compete, even at 50-fold excess over the radioactive ssDNA. A similar experiment was performed using complexes between radioactive dsDNA and the yeast SSB. Nonradioactive dsDNA competed with dsDNA as theoretically expected. Nonradioactive ssDNA competed better than dsDNA, leaving 34% of the complexes intact at equimolar concentration (data not shown) as predicted from the results shown in Figure 3A,B. Nonradioactive ssRNA competed very weakly with dsDNA; even at a 50-fold excess of ssRNA, 52% of the radioactive dsDNA/protein complexes could be trapped on the filter.

Stimulation of the Yeast Strand Exchange Protein by the Yeast SSB. The chromatographic characteristics and the nucleic acid binding properties of the yeast SSB were reminiscent of the properties of the *E. coli* SSB protein. Therefore, we decided to test whether the yeast strand exchange protein was stimulated by the yeast SSB as the *E. coli* RecA protein is stimulated by the *E. coli* SSB. Initial titration experiments indicated that a constant amount of yeast SSB stimulated the strand exchange activity, as assayed by the formation of joint molecules from linear duplex M13mp19 DNA and M13mp19 viral DNA. The overall sigmoidal shape of the titration curve for strand exchange protein remained unchanged from that observed previously (Kolodner et al., 1987), but less strand exchange protein was needed for activity in the presence of yeast SSB (data not shown). This was not due to the presence of strand exchange activity in the SSB, since the yeast SSB alone was found to be completely inactive in the strand exchange assay (data not shown). Figure 4 shows the effect of different amounts of yeast SSB on strand exchange catalyzed by a low, limiting amount of strand exchange protein (0.84 μg/0.3 nmol of ssDNA). The result showed a stimulation with a stoichiometry at maximum stimulation of 1 M_r 34 000 monomer of the yeast SSB per 18 nucleotides of ssDNA (Figure 4). This optimum was independent of the concentration of yeast strand exchange protein used, as a similar value was found using 1.7 μg of yeast strand exchange protein in the assay (not shown). The *E. coli* SSB protein, however, failed to stimulate the yeast strand exchange activity (Figure 4). In fact, at higher concentrations of the *E. coli* SSB protein, the reaction was slightly inhibited.

The rate of joint molecule formation in the presence of limiting concentrations of strand exchange protein was stimulated at least 18-fold in presence of yeast SSB (Figure 5A), as deduced from the initial rate of joint molecule formation during the first 10 min of the reaction. Moreover, the initial lag period seen when suboptimal amounts of strand exchange protein were used (see Figure 5A) was also eliminated in the presence of SSB. When strand exchange protein was not limiting, the rate of the reactions was indistinguishable in the presence or absence of yeast SSB (Figure 5B). By careful titration of the strand exchange protein, we could achieve conditions where the strand exchange activity was completely dependent on the presence of the yeast SSB. Preliminary electron microscopic analysis of the reaction products showed that the same product molecules were formed in reactions stimulated by yeast SSB as in reactions containing only the strand exchange protein [see Kolodner et al. (1987) for examples of these product molecules].

Stimulation of E. coli RecA Protein by Yeast and E. coli SSB. Even though the *E. coli* SSB protein failed to substitute for the yeast SSB in the stimulation of the yeast strand exchange protein, we determined if the yeast SSB could stimulate the *E. coli* RecA protein. Figure 6 shows the results of a titration experiment where the indicated amounts of *E. coli* RecA protein were used in reactions containing no SSB or either the yeast SSB or the *E. coli* SSB present at 1 monomer per 20 nucleotides of ssDNA. The experiment shows that the yeast SSB stimulated RecA equally well as the cognate, *E. coli* SSB.

Discussion

As part of a larger effort to characterize the enzymology of genetic recombination in yeast, we have attempted to identify accessory proteins that stimulate the previously isolated yeast strand exchange protein. Since previous analysis indicated that the *E. coli* RecA protein is stimulated by the *E. coli* SSB, we sought a functional analogue of the *E. coli* SSB

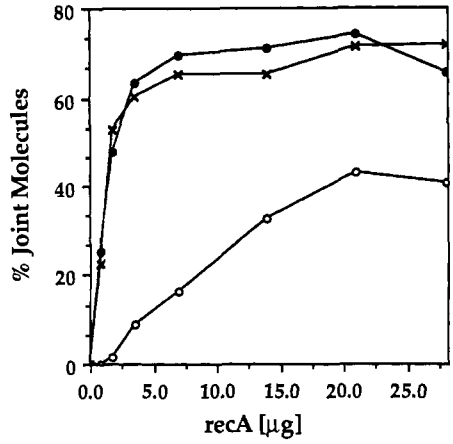

FIGURE 6: Stimulation of *E. coli* RecA protein by yeast and *E. coli* SSB. Strand exchange reaction with the *E. coli* RecA protein were performed as described under Materials and Methods. Each reaction contained the indicated amount of *E. coli* RecA protein and either no SSB (○), 540 ng of yeast SSB (fraction V) (●), or 300 ng of *E. coli* SSB (×).

in yeast. Such a protein has been purified from mitotic yeast cells, and we suspect that by using this assay we might be able to identify additional proteins that stimulate the yeast strand exchange protein.

Genetical and biochemical considerations led Alberts (1984) to propose the involvement of multiprotein complexes in recombination similar to the results obtained in studies on replication proteins. The yeast strand exchange protein is an attractive candidate to play a central role in a multiprotein complex that is involved in recombination. An important question is what other proteins might be involved in such recombination reactions in yeast. In the case of phage λ site-specific recombination, the requirement for the host Fis protein in addition to Xis, Int, and IHF in the excision reaction was noticeable in vitro only when limiting amounts of Xis protein were present (Thompson et al., 1987). It was postulated that the excision reaction under certain conditions should become dependent on the presence of Fis when only limiting amounts of Xis protein are in the cell. Very recently, this has been verified by the demonstration that an *E. coli* Fis-defective mutant is deficient for phage λ excision (R. C. Johnson, personal communication). In the present studies, we identified a protein that stimulated the yeast strand exchange protein in vitro using an assay that contained limiting amounts of strand exchange protein. Our rationale for this approach was that it was likely that cells contain limiting amounts of strand exchange protein considering the high amounts of protein that are required to observe activity in vitro. In analogy to the original studies with *E. coli* Fis protein, we imply that the requirement for the yeast SSB in vitro bears significance for the in vivo situation and think that this is a viable approach to identify proteins that interact with the strand exchange protein. More biochemical and genetical studies will be required to analyze the in vivo role of the identified candidate proteins.

We have purified a protein from mitotic yeast cells that stimulated a cognate strand exchange protein. It consists of an M_r 34 000 polypeptide that qualifies as a ssDNA binding protein for several reasons: (1) The purification protocol shows that the protein had higher affinity for ssDNA than for dsDNA, which is used classically as an initial criterium for the identification of SSBs. In addition, the elution profile of the protein in ssDNA–cellulose chromatography was very reminiscent of *E. coli* SSB and T4gp32. (2) The nucleic acid binding properties of the purified protein demonstrated a higher affinity for ssDNA than for dsDNA or ssRNA. (3) Chase and Williams (1986) have pointed out that HMG proteins are likely to be identified in a search for SSBs. We now have a sequence for 59 amino acid residues of various proteolytically derived fragments of the M_r 34 000 protein. Only three glycine residues were found, suggesting it is very unlikely that the protein is an HMG-type protein, since HMG proteins are distinguished by a high glycine content. Moreover, a database search with these sequences failed to reveal any significant homology with any identified protein. (The amino acid sequencing of the proteolytically derived fragments and the cloning of the corresponding gene by hybridization with appropriate oligonucleotides will be published elsewhere.) (4) The yeast SSB will substitute for *E. coli* SSB in reactions with RecA protein and must share some properties in common with *E. coli* SSB.

The M_r 34 000 protein described here differs in several respects from other previously isolated yeast SSBs (Chang et al., 1975; LaBonne & Dumas, 1983; Jong et al., 1985). First, the protein elutes at a much higher concentration of NaCl during chromatography on ssDNA–cellulose columns. Second, the nucleic acid binding properties are clearly different from the previously identified protein, since the M_r 34 000 protein has low affinity for dsDNA and does not appear to bind to ssRNA. Third, the molecular weight of the SSB characterized in this report differs from the other SSBs. Several possibilities could account for why this protein was not previously identified. First, fraction I in this study was prepared by a gentle lysis procedure that does not entirely disrupt cells. Therefore, less free DNA is generated, which could complex all of the SSB. Second, the protein eluted broadly from 880 to 1500 mM during ssDNA–cellulose chromatography. Therefore, the concentration of the SSB after ssDNA–cellulose chromatography was low. To circumvent this problem, it was necessary to concentrate the fractions and load large volumes on protein gels to detect the SSB protein than with proteins that eluted earlier (see legend to Figure 1). In earlier studies, even if present, the SSB studied here would have been too dilute to visualize by $NaDodSO_4$/PAGE following staining with Coomassie Blue.

The yeast SSB stimulated the cognate mitotic strand exchange protein which has been previously purified in this laboratory (Kolodner et al., 1987; Heyer et al., 1988). The mode of stimulation suggests that the SSB protein acts in a stoichiometric fashion, because 1 protein molecule per 18 nucleotides of ssDNA was required for maximal stimulation. Although the mechanism of stimulation remains unclear at this time, the addition of yeast SSB to suboptimal concentrations of strand exchange protein resulted in a similar rate of joint molecule formation as occurred when a saturating amount of strand exchange protein was assayed in the absence of SSB. Preliminary electron microscopic analysis of the products of strand exchange reactions stimulated by SSB suggests that the same products are generated as in the absence of SSB, but in higher quantities (to be published elsewhere). These product molecules were similar to those formed by saturating amounts of strand exchange protein in the absence of SSB as described before (Kolodner et al., 1987). Kinetic studies showed that the initial lag phase in strand exchange reactions was eliminated and that the rate of joint molecule formation was considerably higher when SSB was added to a suboptimal amount of strand exchange protein. Further analysis will be required to determine whether the stimulation occurred in the initial phase of joint molecules formation or

later in the branch migration phase. Sugino et al. (1988) have reported the stimulation of a meiotic strand exchange protein from yeast by an M_r 20000 protein; however, a detailed analysis of the properties of this stimulatory factor has not been described.

The *E. coli* SSB and other prokaryotic SSBs stimulate the *E. coli* RecA protein in what is believed to be an unspecific manner involving an interaction between SSB and the ssDNA substrates (Cox & Lehman, 1981a; Muniyappa et al., 1984; Egner et al., 1987; Chow et al., 1988). The yeast SSB was also found to stimulate the RecA protein. The titration curves for the RecA protein were indistinguishable when either the cognate *E. coli* SSB or the yeast SSB was used. This suggests that the yeast SSB interacts with ssDNA in a manner that is similar to prokaryotic SSBs. Although the yeast SSB could substitute for the *E. coli* SSB in the stimulation of the *E. coli* RecA, the prokaryotic SSB was, in turn, unable to substitute for the yeast SSB in the stimulation of the yeast strand exchange protein. This suggests that a specific interaction between the yeast SSB and the yeast strand exchange protein may be required for stimulation for strand exchange.

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