Renaturation of DNA by a Saccharomyces cerevisiae Protein That Catalyzes Homologous Pairing and Strand Exchange*

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A protein from mitotic Saccharomyces cerevisiae cells that catalyzes homologous pairing and strand exchange was analyzed for the ability to catalyze other related reactions. The protein was capable of renaturing complementary single-stranded DNA as evidenced by S1 nuclease assays and analysis of the reaction products by agarose gel electrophoresis and electron microscopy. Incubation of the yeast protein with complementary single-stranded DNA resulted in the rapid formation of large aggregates which did not enter agarose gels. These aggregates contained many branched structures consisting of both single-stranded and double-stranded DNA. These reactions required stoichiometric amounts of protein but showed no ATP requirement. The protein formed stable complexes with both single-stranded and double-stranded DNA, showing a higher affinity for single-stranded DNA. The binding to single-stranded DNA resulted in the formation of large protein-DNA aggregates. These aggregates were also formed in strand-exchange reactions and contained both substrate and product DNAs. These results demonstrate that the S. cerevisiae strand-exchange protein shares additional properties with the Escherichia coli recA protein which, by analogy, gives further indication that it might be implicated in homologous recombination.

Genetic recombination is a complex process in which the genetic material is rearranged in a controlled fashion. The molecular mechanism(s) for these processes are unclear, and several competing models (1–3) have been proposed. Common to all models are the following two concepts which are central to our thinking about homologous recombination: (i) the formation of hybrid DNA, and (ii) the presence of crossed-strand structures, the so-called Holliday-junctions. The formation and processing of Holliday-junctions has been extensively documented (4–9), while the formation of hybrid DNA in Saccharomyces cerevisiae is the subject of this communication.

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The pairing of DNA molecules at homologous regions and subsequent exchange of DNA strands results in the formation of joint molecules containing regions of symmetric and/or asymmetric hybrid DNA. The strand-exchange reaction is probably the most extensively studied aspect of the enzymology of recombination. Most of our understanding comes from the pioneering work with the Escherichia coli recA protein, which is involved in recombination, DNA repair, and the regulation of the SOS response (for a review see Ref 10). The recA protein can catalyze the formation of hybrid DNA starting with a variety of different DNA substrates (as reviewed in Refs. 11, 12). Mechanistically, one of the best understood processes is the formation of joint molecules between circular single-stranded DNA and linear double-stranded DNA (13–15, 19, 20). Initially, recA binds stoichiometrically to single-stranded DNA to form an active intermediate (16–18) in the formation of joint molecules. The development of the hybrid DNA ("branch migration") is polar and proceeds in a 3' to 5' direction with respect to the invading strand (19, 20). The bacteriophage T4 uvsX protein has been shown to catalyze strand-exchange reactions in a similar fashion (21).

Proteins that catalyze strand-exchange have also been purified from eukaryotes (22–27). The recA protein from Ustilago maydis catalyzes hybrid DNA formation in the 5' to 3' direction which is in the opposite direction to strand-exchange catalyzed by recA (28). An activity that catalyzes limited strand-exchange in the 5' to 3' direction has been purified from human cells (25). This activity does not require ATP, differing in this respect from the strand-exchange proteins described above.

Recently, our laboratory has purified and characterized a strand-exchange protein from mitotic S. cerevisiae cells (26). This protein shares a number of characteristics with the E. coli recA protein. The reaction requires homologous substrates and stoichiometric amounts of protein, and it shows a cooperative dependence on protein concentration. Joint molecules are formed by displacement of one strand of the linear duplex by the single-stranded circular molecule, and hybrid DNA formation occurs in the 3' to 5' direction, making the S. cerevisiae protein in this respect unique among the eukaryotic strand-exchange activities.

Whereas the involvement of the prokaryotic enzymes (recA, uvsX) in genetic recombination has been demonstrated by genetic evidence, the role of the eukaryotic strand-exchange activities in recombination can only be inferred by analogy to the recA or uvsX proteins. Although the rec1 protein is missing in U. maydis recl strains (29), a recombination deficient mutant, a firm gene-product relationship has not been established. In S. cerevisiae, a direct approach using reverse genetics to clone the gene encoding the strand-exchange protein and to study its in vivo function is now possible.
The renaturation of complementary single-stranded DNA fragments can be considered as the simplest pairwise reaction. The E. coli recA protein was found to support a complemen-
tary single-stranded DNA in an ATP-dependent fashion (30–33). In this paper, we present evidence that the S. cerevisiae strand-exchange protein renatures complementary single-stranded DNA. The reaction was characterized by an S1 nuclease assay, and the products of the reaction were analyzed by agarose gel elec-
trophoresis and electron microscopy. We also report data on the DNA-binding properties of the S. cerevisiae protein and found that the protein aggregates DNA into large complexes.

MATERIALS AND METHODS

Strains—The S. cerevisiae strain BJ 926 a/a, trpl/TRP1, HIS1/ his1, prcl-126/prcl-126, pep3-3/pep3-3, prbl-1122/prbI-1122, can I/ can I was from Dr. D. Hinkle (University of Rochester, NY). The E. coli strain AB259 HfrH, thi-1, rel-1 and bacteriophage M13mp19 were from laboratory stocks.

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 phos-
phate, calf thymus DNA (Type I) were from Sigma.

Nucleic Acids—To purify M13mp19 viral DNA, M13mp19 phage were obtained by standard methods, purified by standard phage purification procedures (26). M13mp19 viral DNA was extracted from the purified phage particles and M13mp19 RFI DNA was purified from infected cells as previously described (26). 2H-Labeled M13 DNA was obtained by growing the cells in 2 liters of Fraser’s medium (34) supplemented with 0.01% thiamine to a density of OD{sub 600} = 0.15. Then 5 ml of [H]thymidine (20 Ci/mmol; Du Pont-New England Nuclear) and adenosine and deoxyadenosine, both to final concentration of 1 mM, were added. The cells were grown until density of OD{sub 600} = 0.7, and then phage were added to a

Assay for DNA Renaturation—Assays were carried out in 15 μl as described for the strand exchange assay (26). The reaction contained 33 mM Tris-HCl, pH 7.5, 13 mM MglCl, 1.3 mM diethiothreitol, 3 mM creatine phosphate, 88 μg/ml bovine serum albumin, 10 units/ml creatine kinase, and 0.15 nmol of HindIII cleaved double-

3H-labeled M13mp19 [H]DNA (37,000 cpm/nmol), which had been
denatured by boiling for 3 min and quenching on ice. After the

RNAse Protection Assay—After the incubation period. Subsequent

5.4 μl of 10% NaDodSO4 was added per 15 μl of renaturation/ protection reaction assay mix after the incubation period. Subsequent

addition of enzyme, reactions were incubated for 5 min at 30 °C, denatured by boiling for 3 min and quenching on ice. After the

samples through GF/C filters, the samples were centrifuged for 10 min in an Eppendorf microcentrifuge at 4 °C, and 0.5 ml of the supernatant was mixed with 4 ml of Aqueous (Du Pont-New England Nuclear) and counted to determine the acid-soluble radioactivity. Under these conditions, the single-stranded DNA was degraded comple-
tely by S1 nuclease, leaving >95% of the radioactivity acid-soluble. Upon omission of S1 nuclease, >95% of the radioactivity was trapped on GF/C filters leaving <5% acid-soluble radioactivity. The S1 nuclease assay using complementary single-stranded substrate DNA is referred to as the DNA renaturation assay. The amount of renatur-
sation is expressed as percent acid-precipitable radioactivity relative to a control where S1 nuclease was omitted.

DNA Binding Assays—Fifteen μl of DNA renaturation reactions were carried out with either 0.15 nmol of single-stranded M13mp19 viral [H]DNA (39,000 cpm/nmol) or 0.15 nmol of double-stranded M13mp19 RFI [H]DNA (37,000 cpm/nmol) and incubated for 5 min at 30 °C. The samples were diluted 33-fold with 500 μl of ice-cold wash buffer containing 33 mM Tris-HCl, pH 7.5, 13 mM MgCl{sub 2}, 1.8 mM diethiothreitol and filtered through KOH-treated nitrocellulose filters (BAbb, 0.45 μm; Schleicher & Schuell) (35). The filters were washed with 1 ml of wash buffer and dried for 10 min under an infrared heating lamp, and then radioactivity was determined as described above.

Electron Microscopy—Ninety μl DNA renaturation reactions were carried through the Protease K digestion step as described above. Then the reactions were chromatographed on a Pasteur pipet columns 3 μm (Bio-Rad) filled with 0.5 μl of 100 mM Tris-EDTA buffer containing 0.1 M NaCl. The DNA containing fractions were microphotographed for electron microscopy by the formamide technique essentially as described (36). Alternatively, 90 μl DNA renaturation reactions were carried through the Protease K step, and then the samples were electrophoresed through a 0.8% agarose gel. The renaturation product band and the corresponding region in the control lane (no enzyme added) were eluted by the "freeze-squeeze" technique (37), and the DNA was used for electron microscopy.

DNA Aggregation—Aggregation of DNA by the yeast strand-ex-
change protein was assayed as described (36, 39). Briefly, 90-μl
DNA samples were centrifuged at 100,000 g in an Eppendorf microcentrifuge. Three sequential aliquots of 9 μl were removed from the supernatant. The remaining 3 μl and the pellet were suspended in 200 μl of water. The amount of DNA in the total supernatant (30 μl) was estimated from the average radioactivity of the first 2 aliquots of the super-
natant. The amount of radioactivity in the pellet was corrected for the presence of 3 μl of supernatant.

RESULTS

The Yeast Strand-Exchange Protein Renatures Complement-
ary Single-stranded DNA—Heat-denatured, linear, double-
stranded M13mp19 DNA, incubated with the S. cerevisiae strand-exchange protein (1 M, 132,000 polypeptide/26 nucleo-
tides of single-stranded DNA), was quantitatively converted
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to a high molecular weight form that remained at the top of an agarose gel (Fig. 1A, lane 9). The product was not formed in the absence of protein (Fig. 1A, lane 3). The generation of this product was fully dependent on the presence of MgCl₂ (Fig. 1A, lane 4) but did not require dithiothreitol (lane 5) or ATP (lane 7). The reaction was completely inhibited by 200 mM NaCl (Fig. 1A, lane 8), but the addition of 5 mM N-ethylmaleimide had little or no effect (Fig. 1A, lane 6). In these experiments the substrate DNA (linear, single-stranded DNA) was slightly fragmented due to the heat denaturation step and was not easily seen because it was a diffuse band that did not stain intensely with ethidium bromide. The reaction requirements were identical when lower amounts of protein (1 M, 132,000 polypeptide/264 nucleotides of single-stranded DNA) were used which resulted in only partial renaturation of the DNA as judged from the S1 assay (data not shown). The formation of this product was dependent on complementary DNA. When noncomplementary single-stranded M13mp19 viral DNA was used as substrate (Fig. 1B, lanes 4–7) instead of complementary single-stranded DNA (Fig. 1A), no high molecular weight product DNA was formed. This control also excludes the possibility that the product that remained at the top of the gel represented a protein:DNA complex that somehow withstood the NaDodSO₄/EDTA/Proteinase K treatment. Even at higher protein concentrations (Fig. 1B, lane 7), no ethidium bromide stainable material was detected at the top of the gel in experiments with noncomplementary single-stranded DNA. Since the reaction product of the renaturation reaction did not enter the gel, quantitation of the reaction by densitometry of the gel was not possible. Therefore, we attempted a quantitative approach of the reaction using an S1 nuclease assay.

Characterization of the DNA Renaturation Reaction—Since double-stranded DNA is resistant to S1 nuclease, it is possible to accurately quantify the amount of renaturation by measuring the extent to which complementary single-stranded DNA becomes S1 resistant. A protein titration curve is illustrated in Fig. 2 (×). We found that complementary single-stranded DNA was converted to an S1 resistant form but that the interpretation of the data was complicated by the fact that a portion of the S1-resistant DNA could be explained by the protection of single-stranded DNA from degradation by S1 nuclease (Fig. 2A and discussed in detail below). Therefore in Fig. 2A the curve labeled renaturation is in fact the sum of renaturation and protection explaining why 100% of the DNA could be rendered S1 resistant.

We developed conditions under which addition of NaDodSO₄ prior to digestion with S1 nuclease prevented the protection of single-stranded DNA (Fig. 2B). All of the data using the S1 digestion assay to quantitate renaturation that is discussed below (Fig. 2B, Fig. 3, and Table I) were obtained with the assay utilizing NaDodSO₄. The reaction showed that maximal renaturation occurred at 200 ng of protein/0.15 nmol of single-stranded DNA, or about 1 M, 132,000 polypeptide/100 nucleotides (assuming that all of the protein molecules were active). No more than 70% renaturation was observed in any experiment (Fig. 2B, Fig. 3) which is consistent with the structure of the renaturation products observed by electron microscopy (see below). Higher protein concentrations were not inhibitory, which is in contrast to the strand-exchange reaction (26). The renaturation reaction was fast; within 2 min of incubation at 30 °C, most of the product was already formed (Fig. 3). The data also show that the protein did not act catalytically, since the products of the reaction reached limits proportional to the initial concentration of strand-exchange protein with identical kinetics. Thus at half the protein concentration, the maximum of renaturation was half; at 100 ng maximally 48% of the DNA was S1 resistant, at 50 ng 19%, and at 25 ng 8%. The requirement for different components in the reaction was determined using the S1 nuclease assay (Table I). DNA renaturation required Mg²⁺. The reaction was inhibited by 200 mM NaCl, but not by 5 mM N-ethylmaleimide. There was also no requirement for ATP or an ATP regeneration system. Results obtained with the S1 nuclease assay and the agarose gel electrophoresis
protein concentrations. Reactions were carried out as described under "Materials and Methods" and incubated at 30 °C. Fifteen-μl aliquots were removed and processed by digestion with S1 nuclease in the presence of NaDodSO4 at the indicated times. Reactions contained either no protein (●); 400 ng (○); 200 ng (╳); 100 ng (■); 50 ng (▲); 25 ng (□); and 12.5 ng (△).

**TABLE I**

*Reaction requirements of DNA renaturation*

| Relative activity | % |
|-------------------|---|
| Complete reaction mixture | 100 |
| - Protein | 15 |
| - Mg2+ | 18 |
| - Dithiothreitol | 123 |
| - Dithiothreitol, +5 mM N-ethylmaleimide | 134 |
| - ATP regeneration system, - ATP | 136 |
| +200 mM NaCl | 7 |

* Omission of creatine phosphokinase and creatine phosphate.

assay were completely consistent with one another.

**Structure of DNA Renaturation Products**—The DNA renaturation products were examined by electron microscopy. Complementary single-stranded DNA that had been incubated with the strand-exchange protein was found predominantly (>95% of the mass) in the form of complex aggregates (Fig. 4, A and B). The aggregates consisted of double-stranded and single-stranded stretches of DNA and were resistant to the NaDodSO4/EDTA/Proteinase K treatment. No differences were found when the reaction products were purified from an agarose gel and then spread for electron microscopy. As a reference 5 ng of circular single-stranded M13mp19 viral DNA was cospread along with the reaction products. A, a typical reaction product obtained from a reaction containing Fraction V where the product was gel purified and mounted for electron microscopy. As a reference 5 ng of circular single-stranded M13mp19 viral DNA was cospread along with the reaction products. B, a typical reaction product obtained from a reaction containing Fraction V where the reaction was chromatographed on an Agarose A5m column and then mounted for electron microscopy. As a reference 5 ng of circular single-stranded M13mp19 viral DNA was cospread along with the reaction products. C, control spreading for B, where the reaction mixture contained no protein.

**FIG. 3.** Time course of DNA renaturation at various protein concentrations. Reactions were carried out as described under "Materials and Methods" and incubated at 30 °C. Fifteen-μl aliquots were removed and processed by digestion with S1 nuclease in the presence of NaDodSO4 at the indicated times. Reactions contained either no protein (●); 400 ng (○); 200 ng (╳); 100 ng (■); 50 ng (▲); 25 ng (□); and 12.5 ng (△).

**FIG. 4.** Electron microscopic analysis of DNA renaturation products. Reaction mixtures (90 μl) were incubated for 5 min at 30 °C and contained either 900 ng of Fraction V or no protein. A, a typical reaction product obtained from a reaction containing Fraction V where the product was gel purified and mounted for electron microscopy. As a reference 5 ng of circular single-stranded M13mp19 viral DNA was cospread along with the reaction products. B, a typical reaction product obtained from a reaction containing Fraction V where the reaction was chromatographed on an Agarose A5m column and then mounted for electron microscopy. As a reference 5 ng of circular single-stranded M13mp19 viral DNA was cospread along with the reaction products. C, control spreading for B, where the reaction mixture contained no protein.
DNA Binding Properties of the S. cerevisiae Strand-Exchange Protein—The protein formed stable complexes with both single-stranded and double-stranded DNA. Fig. 5 shows the result of a protein titration experiment measuring the binding of stable protein:DNA complexes to nitrocellulose filters. Maximal complex formation between Fraction V and single-stranded DNA was observed at about 100 ng of protein/0.15 nmol of single-stranded M13mp19 viral DNA. The affinity of Fraction V for double-stranded DNA was much lower than for single-stranded DNA (Fig. 5). Maximal complex formation occurred at about 375 ng of Fraction V/0.15 nmol of double-stranded DNA. No further increase in complex formation was observed at about 100 ng of Fraction V in reactions with single-stranded DNA. The affinity of Fraction V for double-stranded DNA was much lower than for single-stranded DNA (Fig. 5). Maximal complex formation occurred at about 375 ng of Fraction V/0.15 nmol of double-stranded DNA. No further increase in complex formation was observed at about 100 ng of Fraction V/0.15 nmol of double-stranded DNA. The reaction requirements for protein:DNA complex formation were determined, and the results are presented in Table II. The formation of stable protein:DNA complexes did not require Mg²⁺ and was only partially inhibited by N-ethylmaleimide. There was also no requirement for ATP or an ATP regeneration system. The salt stability of the protein:DNA complexes was measured as described in “Materials and Methods.” Reaction mixtures (15 μl) were incubated at 5 min at 30 °C and contained the indicated amount of Fraction V. The formation of stable complexes was measured as described under “Materials and Methods.” Reactions with single-stranded DNA (x) and with double-stranded DNA (■). The nonspecific DNA binding to nitrocellulose filters in the absence of protein was subtracted from the values presented in the figure.

### Table II

| Relative binding | ssDNA | dsDNA | % |
|------------------|-------|-------|---|
| Complete reaction mixture | 100   | 100   | 100 |
| Protein          | 2     | 2     | 20 |
| Mg²⁺             | 96    | 109   | 96 |
| Dithiothreitol   | 96    | 106   | 96 |
| Dithiothreitol, +5 mM N-ethylmaleimide | 75    | 79    | 79 |
| ATP regeneration system, ATP | 104   | 114   | 114 |
| +200 mM NaCl     | 73    | 61    | 61 |

* Omission of creatine phosphokinase and creatine phosphate.

A, time course of DNA aggregation and S1 protection. Reactions (660 μl) containing 2.2 μg of noncomplementary M13 viral DNA were incubated with 13.4 μg of Fraction V at 30 °C. At the indicated time points 2 aliquots of 60 μl were removed. The first aliquot was subjected to the S1 protection assay, determining the amount of DNA that was sensitive to digestion by S1 nuclease in the absence of NaDodSO₄. Control reaction without protein, (x); reaction with protein, (■). The second aliquot was centrifuged for 4 min and the radioactivity in the supernatant and in the pellet was determined as described (38, 39). This gave the amount of the DNA present in aggregates. Control reaction without protein, (A); reaction with protein, (C). B, DNA aggregation during the strand-exchange reaction. Reactions (135 μl) containing 900 ng of EcoRI cleaved M13mp19 double-stranded DNA and 450 ng of circular M13mp19 viral [³H]DNA were incubated with 13.4 μg of Fraction V for 20 min at 30 °C. Aliquots of 30 μl were removed and analyzed. Lanes 1 and 2, 50 μl were centrifuged for 4 min and processed as described under “Materials and Methods.” The pellet was suspended in 25 μl 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, treated with NaDodSO₄/EDTA/Protease K and analyzed by electrophoresis on an agarose gel. This represents the DNA present as aggregates. lane 1, reaction with protein; lane 2, reaction without protein. Lanes 3 and 4, 50 μl was treated directly with NaDodSO₄/EDTA/Protease K and analyzed directly by agarose gel electrophoresis as in the standard strand-exchange assay (26). Lane 3, reaction with protein; lane 4, reaction without protein. Additional aliquots were assayed for DNA aggregation and S1 nuclease sensitivity as described in the text.

Aggregation of DNA by the Yeast Strand-exchange Protein—As discussed below, we reasoned that the protection of noncomplementary, single-stranded DNA from S1 nuclease digestion by the yeast strand-exchange protein was not mediated by simple covering of the DNA by the protein as in a footprint type of protection. Therefore, we tested whether the protein sequesters DNA in large aggregates rendering the DNA S1 resistant. Incubation of the yeast strand-exchange protein with noncomplementary single-stranded DNA resulted in the formation of large protein:DNA aggregates, which sedimented at greater than 10,000 S (38). The formation of these aggregates had the same kinetics as the protection of the DNA from S1 nuclease digestion (Fig. 6A) measured by simple covering of the DNA by the protein as in a footprint type of protection.
ured as a decrease in the amount of S1-sensitive DNA present. The addition of NaDodSO₄ to 0.1% completely disrupted these aggregates (data not shown). This suggests that it is the formation of these large aggregates which renders the DNA inaccessible to S1 nuclease.

In a strand-exchange reaction, the protein coaggregated homologous, linear double-stranded and circular single-stranded DNA, as illustrated in Fig. 6B. The DNA in the reaction was quantitatively aggregated and present in the pellet (compare lane 1 with lane 3). The small amount of substrate DNA (linear double-stranded and circular single-stranded DNA) in the control reaction (lane 2) was due to the presence of the residual 3 μl of supernatant in the pellet fraction. Assays of parallel aliquots showed that the ³H-labeled single-stranded DNA was protected from S1 nuclease digestion. Furthermore, the aggregation assay confirmed that all radioactivity was present in the pellet leaving no radioactivity in the supernatant. Interestingly, as seen in Fig. 6B, lane 1, the aggregates contain substrate DNA (linear double-stranded and circular single-stranded DNA) as well as product DNA (joint molecules).

DISCUSSION

We have shown that the S. cerevisiae strand-exchange activity renatures complementary single-stranded DNA. Three independent pieces of evidence support this notion: 1) the yeast activity forms a new DNA species on agarose gels, after incubation with complementary single-stranded DNA, which is reminiscent of the renaturation products formed by the E. coli recA protein (30); 2) the yeast activity transforms complementary single-stranded DNA into an S1 nuclease-resistant form under conditions where protection of noncomplementary DNA was eliminated by treatment with NaDodSO₄, which has been a classic definition of DNA renaturation, (30–33); and 3) renaturation products, when examined by electron microscopy, were shown to be large aggregates containing double-stranded DNA which were very similar to the products formed by recA (30).

The DNA renaturation reaction can be regarded as the simplest model for the formation of hybrid DNA and will be compared in the following to the strand-exchange reaction performed by the same activity. All the assays described in this paper were performed under strand-exchange conditions (as used in Ref. 26) to simplify this comparison. Both reactions required stoichiometric amounts of proteins, a different stoichiometry is required, however. Maximum strand-exchange required 1 monomer/12–14 nucleotides of single-stranded DNA (26), whereas maximum DNA renaturation was achieved by 1 monomer/100 nucleotides. This is more protein than expected from a mechanism in which the yeast protein simply provides a nucleation point for DNA renaturation. The reaction requirements are identical with the notable exception that N-ethylmaleimide inhibits strand-exchange completely but has no effect on DNA renaturation. This suggests that the molecular mechanisms for both reactions are not identical and that there is a different requirement for sulfhydryl-group(s).

The yeast strand-exchange activity forms stable complexes with single-stranded and double-stranded DNA. It has a high affinity for single-stranded DNA, whereas the affinity to double-stranded DNA is much lower. It is not clear at this point whether the yeast strand-exchange protein binds first to single-stranded DNA in the strand-exchange reaction as was shown for the recA protein (16–18). The stability of the protein:single-stranded DNA complexes at 200 mM NaCl (more than 70% of the complexes persist) suggests that the sensitivity of the DNA renaturation and strand-exchange reaction to NaCl at this concentration is not due to the simple disruption of the protein:single-stranded DNA complexes.

The yeast strand-exchange activity was found to protect single-stranded DNA from digestion by S1 nuclease at high protein concentrations. Since the S1 nuclease assay is widely used to demonstrate and quantitate DNA renaturation, it should be pointed out that this protection from S1 nuclease digestion might be a source of artifacts in DNA renaturation assays where a control with noncomplementary single-stranded DNA has not been performed or where the protein has not been dissociated from the DNA prior to S1 digestion. The significant protection of single-stranded DNA at a stoichiometry of 1 M, 132,000 polypeptide/100 nucleotides suggests that there is not enough protein in the reaction to completely cover the DNA for a footprint-type of protection. The strand-exchange reaction, for comparison, requires 1 M, 132,000 polypeptide/12–14 nucleotides. In a time course of S1 protection at even lower protein concentrations (1 M, 132,000 polypeptide/440 nucleotides) the protection effect was low at 5 min (19%) but steadily climbed to a maximum of 78% at 40 min. This slow time course also suggests that the S1 protection is not mediated solely by simple binding of the protein to DNA because binding to DNA as measured in the nitrocellulose filter binding assay is completed within 2 min (data not shown). This interpretation is strengthened by the observation that the activity forms fast sedimenting aggregates with single-stranded DNA at high protein concentrations which could render the single-stranded DNA inaccessible to S1 nuclease. This is also suggested by the parallel time course of the DNA aggregation and S1 protection reactions. Additionally, these aggregates were also found to be sensitive to detergent as was the S1 protection. This aggregate formation is reminiscent of the network formation of DNA and recA protein that was discovered by Radding and co-workers (38, 39). The yeast strand-exchange activity will aggregate DNA under strand-exchange conditions. Since we find that the DNA is quantitatively aggregated, we hypothesize that this is an early step in the strand-exchange reaction. It is clear that these aggregates are actively undergoing strand-exchange since their formation is complete within 5 min, whereas strand-exchange proceeds in a linear fashion for 60 min (26). This view is supported by the observation that product DNA (joint molecules) is present in the aggregates. Reconstitution experiments with active intermediates will be needed to prove whether the aggregates are obligatory intermediates for the strand-exchange reaction.

The additional properties of the S. cerevisiae strand-exchange activity reported here are similar to properties of previously characterized strand-exchange proteins, most notably to the E. coli recA protein. Thus far the only significant difference between the yeast protein and recA is the lack of an ATP requirement by the yeast activity for any of the reactions studied. A possible explanation for this is discussed in Kolodner et al. (26). Due to the lack of genetic evidence we have to rely on these analogies to the recA protein as an initial indication that this S. cerevisiae activity is involved in homologous recombination. Recently, we have cloned a gene that potentially encodes the strand-exchange protein* and are using the reverse genetic approach available in this organism to establish a gene-protein-function relationship.

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