During purification of a strand exchange activity from Schizosaccharomyces pombe using the three-strand reaction of double-stranded linear and circular single-stranded DNA, we identified p190/210 as an activity that stimulated the strand exchange activity of p140\textsuperscript{iso} by about 10-fold. The accompanying report (Käslin, E., and Heyer, W.-D. (1994) J. Biol. Chem. 269, 0000--0000) described the purification and characterization of p140\textsuperscript{iso}, likely to be the S. pombe homolog of the Saccharomyces cerevisiae strand exchange protein p175\textsuperscript{iso}. Here, we report the purification of p190/210 from S. pombe cells and its identification as fatty acid synthase (FAS). S. pombe FAS (p190/210) binds to single-stranded and double-stranded DNA, leading to condensation of DNA into large aggregates. In addition, it is capable of renaturing complementary single-stranded DNA. Besides stimulating the strand exchange activity of p140\textsuperscript{iso}, FAS (p190/210) itself exhibits strand exchange activity provided the double-stranded substrate has single-stranded tails. We propose a probable mechanism for the action of FAS (p190/210) during DNA strand exchange in vitro. Since FAS (p190/210) is highly unlikely to have a role in homologous recombination in vivo, we discuss the implications of our data on the interpretation of other homologous pairing and strand exchange proteins purified from eukaryotes using this or similar assays.

Since hybrid DNA is known to be a central intermediate in homologous recombination, efforts have been made to purify activities that promote strand exchange and hybrid DNA formation. A prominent in vitro assay is the three-strand reaction with a circular ssDNA molecule and a homologous linear dsDNA molecule as model substrates. Activities that form joint molecules in the three-strand reaction have been purified from prokaryotes as well as from eukaryotes (reviewed in Ref. 1; see introduction of accompanying article, Ref. 2). In the accompanying paper (2), we describe the purification and characterization of p140\textsuperscript{iso}, a Schizosaccharomyces pombe exonuclease which exhibits strand exchange activity in vitro. p140\textsuperscript{iso} is a member of a group of eukaryotic strand exchange proteins with associated exonuclease activity comprising p175\textsuperscript{iso} from Saccharomyces cerevisiae (3), Rrl from Drosophila melanogaster (4), and HPP1 from humans (5). These systems might be similar to the Escherichia coli RecE and λRed pathways, where the in vivo roles of the involved proteins have been established (6).

For some of the strand exchange activities, factors have been purified which stimulate the strand exchange activity (7--9). In this paper we report the purification and characterization of an S. pombe protein, p190/210, which stimulates p140\textsuperscript{iso} (2) in the strand exchange assay. We identified p190/210 as the two subunits of S. pombe fatty acid synthase (FAS).

Fatty acid synthases are responsible for the de novo synthesis of saturated long chain fatty acids. In eukaryotes, the enzyme is localized in the cytoplasm or in the mitochondria. In lower fungi as S. cerevisiae or Penicillium patulum, the homologue is organized in a αβ, three-enzyme complex exhibiting eight enzymatic activities. In S. cerevisiae, the α subunit, a 228-kDa polypeptide, is encoded by the FAS2 gene and the β subunit (206 kDa) by FAS1 (10--12). A striking co-linearity in both, their nucleotide sequence and their component enzyme sequence, has been observed between the S. cerevisiae and the Yarrowia lipolytica FAS1 genes (12) and between the S. cerevisiae and the P. patulum FAS2 genes (13). Thus, the FAS organization in lower fungi is supposed to be conserved.

The finding that FAS stimulates the in vitro strand exchange activity of p140\textsuperscript{iso} raises some doubts on the specificity of the three-strand reaction used in this work. We discuss possible mechanisms how FAS might act in the assay. In addition, possible implications for strand exchange activities and their stimulatory factors, which have been purified with this assay, are discussed.

**EXPERIMENTAL PROCEDURES**

**Strains, Enzymes, Chemicals, and Nucleic Acids—**Strains, enzymes, chemicals, and nucleic acids are described in Käslin and Heyer (2). T7 gene 6 exonuclease resected substrate was made as follows: Xho1-digested M13mp19 dsDNA was 3' labeled with T4 DNA polymerase. 0.5 μg of this DNA was then incubated in a 50-μl reaction with 0.5 unit of T7 gene 6 exonuclease (U. S. Biochemical Corp.) as recommended by the supplier for 1 or 2 min at 37 °C. The reaction was immediately stopped by addition of 2 μl of 0.5 μg/ml EDTA and subsequent incubation at 75 °C for 10 min. Under these conditions, we estimate 100--200 nucleotides released per 5'-end based on the specific activity of the enzyme. Then the DNA was phenol-extracted, ethanol-precipitated, and resuspended in TE.

**Assay for Strand Exchange, Assay for DNA Renaturation, and Electron Microscopy—**These three assays are described in Käslin and Heyer (2). In the strand exchange assay, 6% PEG has been added where indicated. This amount was determined by titration to be optimal.

**Assay for DNA Binding—**DNA binding has been measured essentially as described (14). The amount of bound radioactivity was corrected for unspecific binding of DNA to the filter in the absence of protein (<3%).

**Assay for DNA Aggregation—**DNA aggregation was assayed essentially as described (9, 14). 30-μl reactions were carried out for 2 min at 30 °C and then centrifuged for 5 min at 12,000 rpm in the Microfuge. Three 9-μl aliquots were removed sequentially from the supernatant and added to 100 μl of 0.1% SDS. The remaining supernatant and the pellet were then suspended in 100 μl of 0.1% SDS. The amount of DNA in the supernatant was calculated from the amount of radioactivity present in the first two aliquots of the supernatant. The amount of DNA
in the pellet was corrected for the presence of 3 μl of supernatant.

**Assay for Fatty Acid Synthase Activity**—FAS activity was determined using a spectrophotometric assay measuring activity as oxidation of NADPH (15). The blank rate of NADPH oxidation was subtracted from the rate observed in the presence of substrate. One milliunit is defined as the amount of enzyme causing a reduction of 0.006 absorbance unit measured at 344 nm in 1 min.

**Purification of p190/210**—All the buffers and conditions to purify p190/210 were as described for the purification of p140<sup>med</sup> (2). p190/210 was purified from vegetatively growing S. pombe cells by assaying for stimulation of the strand exchange activity of p140<sup>med</sup> (2). For this purpose, each strand exchange assay, including 100 ng of p140<sup>med</sup> (fraction V of Ref. 2), was supplemented with different amounts (1, 2, and 5 μl) of fractions eluting from the chromatography columns. As p140<sup>med</sup> and p190/210 co-purify on DEAE-cellulose and ssDNA-cellulose, these first steps of the purification were the same as described for p140<sup>med</sup> (2).

The reconstitution of the strand exchange assay was performed starting with the fractions eluting from the phosphocellulose column. In chromatography on phosphocellulose, the activity eluted around 300 mM NaCl, and active fractions were pooled to give fraction IV (16 ml; 0.1 mg/ml). Conductivity of fraction IV was brought to that of buffer A/150 mM NaCl by dilution with buffer A. The diluted fraction IV was loaded on Mono Q (HR 5/5, Pharmacia) at a flow rate of 0.5 ml/min. After washing the column with 5 ml of buffer A, a 12-ml gradient with 150–600 mM NaCl in buffer A was applied, and 0.4-ml fractions were collected. Active fractions eluted at about 215 mM NaCl and were pooled to give fraction V (3.2 ml; 0.2 mg/ml). The conductivity of this fraction was again adjusted to buffer A/100 mM NaCl. This fraction was loaded on a heparin-agarose column (0.63 cm<sup>2</sup> x 8 cm, Sigma) at a flow rate of 10 ml/h. A 40-ml gradient from 100–600 mM NaCl in buffer B was applied, and 0.4-ml fractions were collected (see Fig. 1). Active fractions eluting around 360 mM NaCl were pooled and dialyzed against buffer B containing 20 mM Tris·HCl, pH 7.5, 0.1 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride, 100 mM NaCl, and 60% (w/v) glycerol to give fraction VI (5.8 ml; 0.06 mg/ml). Fraction VI was used throughout except for protein sequencing.

**Protein Sequencing**—The NH<sub>2</sub>-terminal sequence has been determined as follows: 200 μl of p190/210 (fraction V; 200 ng/ml) were dialyzed against 10 mM Tris·HCl, pH 7.5, 5% glycerol, 50 mM NaCl, 1 mM EDTA, and 1 mM DTT for 2 h at room temperature. The protein solution was fractionated on a 5% SDS-polyacrylamide gel. After equilibration of the gel in transfer buffer (50 mM boric acid, 50 mM Tris base) for 1 h, the proteins were transferred onto Immobilon P (Millipore, according to manufacturer’s recommendations) by electroblotting (1 h, 0.5 mA) using a Hoefer TE22. The proteins on the blot were detected with 0.1% Amido Black 10% acetic acid/50% methanol. A pulsed-liquid Sequencer (model 477A, Applied Biosystems) was used for NH<sub>2</sub>-terminal sequencing.

**Miscellaneous Methods**—Where necessary, proteins were diluted in 20 ml Tris·HCl, pH 7.5, 1 mM DTT, 0.5% glycerol, 0.5 mg/ml BSA. Protein concentration was measured using the method of Bradford (17). As a standard, BSA (fraction V, Sigma) was used. Protein samples were analyzed on SDS-polyacrylamide gel electrophoresis according to Ref. 18 using 0.75-mm-thick gels in a Mighty small apparatus (Hoefer).

**RESULTS**

**Identification and Purification of p190/210 from Mitotic S. pombe Cells**—During the purification of p140<sup>med</sup>, a loss of specificity in the strand exchange reaction (see Fig. 11 for a schematic representation of the assay) was observed after chromatography on phosphocellulose. This was taken as an indication for a multicomponent system whose components were separated during the purification. Complementing the fractions from the phosphocellulose containing p140<sup>med</sup> with other fractions from this column, which showed no strand exchange activity on their own, uncovered a hypothetical stimulatory factor which eluted at about 300 mM NaCl from phosphocellulose. We used this reconstitution assay to purify this possible stimulatory factor, which we called p190/210. As the stoichiometry plays an important role in the strand exchange reaction (reviewed in Refs. 19 and 20), we routinely assayed different amounts of the eluting fractions. Fig. 1, panel A, shows the elution of p190/210 during heparin-agarose chromatography, the last purification step, by SDS-polyacrylamide gel electrophoresis analysis. In Fig. 1, panel B, the reconstitution of the strand exchange activity is shown. The level of joint molecule formation does not reach the maximum level seen with optimal concentrations of p140<sup>med</sup> and p190/210, which is seen in the 3rd lane of Fig. 1, panel B (100 ng (710 fmol; 23.6 nM) of p140<sup>med</sup>, fraction V (2); 60 ng (300 fmol; 10 nM) of p190/210 fraction VI). This is most likely due to suboptimal protein concentrations or due to increased NaCl concentrations. p190/210 elutes at about 360 mM NaCl from the heparin-agarose column. The reconstitution assay in which 5 μl of every fraction was assayed (instead of only 2 μl as in Fig. 1B) showed a weaker stimulation over all fractions (data not shown), indicating that the additional salt is interfering with the strand exchange assay. Fig. 2A summarizes the purification of p190/210 in a Coomassie-stained 8% SDS-polyacrylamide gel. p190/210 refers to the apparent subunit molecular weight of the two peptides present in equimolar amounts in this fraction. These two peptides showed perfect co-elution over all chromatographic steps employed. We judged that p190/210 represent more than 95% of the protein present in this fraction.

**p190/210 Is the S. pombe Fatty Acid Synthase**—In order to identify the proteins present in fraction VI, we attempted to determine the NH<sub>2</sub>-terminal amino acid sequence of the two peptides. We received an unambiguous sequence of 20 amino acids for p190, the NH<sub>2</sub> terminus of p210 was apparently blocked. A data bank search with these 20 amino acids of p190 revealed a homology to the NH<sub>2</sub> terminus of the FAS2 gene.
products of P. patulum and S. cerevisiae with 19 out of 20 and 17 out of 20 identical amino acids, respectively (Fig. 3). In yeast, fatty acid synthase (FAS) is an αβ heteromultimeric, multifunctional complex whose subunits are encoded by two genes, FAS1 (subunit β) and FAS2 (subunit α) (10–12). The FAS1 and FAS2 genes encode a protein of 228-kDa and 205-kDa molecular weight, respectively. To ascertain that p190/210 represents S. pombe FAS, we made two more experiments. First, p190/210 cross-reacted with an anti-S. cerevisiae-FAS serum recognizing both subunits (Fig. 2B, lane 2). Although it is difficult to estimate the molecular weight in this range, the S. pombe FAS subunits had a slightly higher mobility, indicating a slightly lower molecular weight than the two subunits of the S. cerevisiae protein (Fig. 2B, lanes 2 and 3). Second, p190/210 catalyzed the oxidation of NADPH to NADP in an assay for FAS holoenzyme activity (Fig. 4). The calculated specific activity for p190/210, determined in the linear decrease in ΔA₃₄₀, between 100 and 300 s, was 2130 milliunits per mg. As a comparison, the co-assayed S. cerevisiae FAS has a specific activity of 1030 milliunits per mg. Both proteins, p190 and p210, apparently formed a complex co-sedimenting with a sedimentation coefficient greater than that of ferritin (17.6 S; 100 μg; III, 0.5 μg; IV, 0.6 μg; V, 0.6 μg; VI, 0.3 μg). Molecular mass markers (high range, Bio-Rad) and their molecular masses (kDa) are: myosin (200), β-galactosidase (116), phosphorylase B (97), BSA (66), and ovalbumin (45); each 0.5 μg per lane. B, Western blot of p190/210 and S. cerevisiae FAS. Lane 1, molecular weight markers (high range, Bio-Rad, see above) stained with Amido black. Lanes 2 and 3, Western blot with anti-S. cerevisiae FAS total serum; lane 2, 360 ng of p190/210 (fraction VI; lane 3, 360 ng of S. cerevisiae FAS holoenzyme.

The strand exchange stimulatory activity of p190/210 is unlikely to be due to a minor contaminant in this fraction. Proteins that lead to aggregation of DNA (see below) need to be present in high stoichiometric amounts (7, 9, 14, 21). Since the preparation is judged to be more than 95% pure, a minor contaminant is unlikely to amount to the required concentration. In addition, p190/210 and strand exchange stimulatory activity co-eluted perfectly over three highly diverse chromatographic steps (phosphocellulose, Mono Q FPLC, and heparin-agarose) making it highly unlikely that a contaminant persistently co-eluted with p190/210. Nevertheless, we designed an additional experiment to exclude the possibility of a minor co-eluting contaminant. As already mentioned above, we knew from previous experiments that p190/210 chromatographed on a gel filtration column as a large complex with a Stokes radius of more than 96.6 Å. We tested fractions from a small gel filtration column to determine the mode of action of p190/210 in the strand exchange assay. We wanted to determine the mode of action of p190/210 in the in vitro strand exchange assay. p190/210 exhibited neither exonuclease activity on ssDNA or on dsDNA nor did it alter the exonuclease activity of p140/110 (data not shown). Using linear dsDNA substrates produced by a restriction endonuclease, p190/210 exhibited no strand exchange activity, forming no joint molecules (Fig. 5A, lane 1). Comparison of lanes 2 and 3 in Fig. 5A gives a direct estimate of the stimulation of p190/210 in the strand exchange assay with p140/110. In lane 3, p190/210 had been heat-denatured prior to the experiment. The remaining strand exchange activity represented the level of joint-molecule-forming activity seen with p140/110 alone (2). Optimal strand exchange stimulation was achieved with 60 ng (300 fmol; 10 nm) of p190/210 (about 150 fmol of each peptide) which is 1 molecule of p190 and p210 per 100 nucleotides of ssDNA or 100 base pairs of dsDNA assuming equimolar representation in the preparation (Fig. 2A). This experiment also showed that the stimulatory effect of p190/210 was a property of the intact protein and was not mediated by merely adding protein.

The next question we addressed was whether the functions of p140/110 and its stimulatory factor, p190/210, can be separated in the strand exchange assay. After pretreating the linear duplex DNA with p140/110 (under conditions where the exonuclease activity of p140/110 was active, i.e. in the presence of Mg²⁺; Ref. 2) and subsequent extraction (this substrate is called “P” for pretreated), the strand exchange assay was performed and

**Fig. 2.** A, electrophoretic analysis and summary of the p190/210 purification. Coomassie-stained 8% SDS-polyacrylamide gel with fractions numbered as described under "Experimental Procedures": I, 50 μg; II, 50 μg; III, 0.5 μg; IV, 0.6 μg; V, 0.6 μg; VI, 0.3 μg. Molecular mass markers (high range, Bio-Rad) and their molecular masses (kDa) are: myosin (200), β-galactosidase (116), phosphorylase B (97), BSA (66), and ovalbumin (45); each 0.5 μg per lane. B, Western blot of p190/210 and S. cerevisiae FAS. Lane 1, molecular weight markers (high range, Bio-Rad, see above) stained with Amido black. Lanes 2 and 3, Western blot with anti-S. cerevisiae FAS total serum; lane 2, 360 ng of p190/210 (fraction VI; lane 3, 360 ng of S. cerevisiae FAS holoenzyme.

**Fig. 3.** Comparison of the NH₂-terminal amino acid sequence of p190 with the deduced NH₂ terminus of the FAS2 gene products of P. patulum and S. cerevisiae. Homology search and alignment was made using the gap program of the GCG package Version 7.1 (53) with EMBL/GenBank release 35.0 and SwissProt release 25.0.

**Fig. 4.** FAS activity of p190/210. 8 μg of S. cerevisiae FAS (○) and 1.2 μg of S. pombe p190/210 (■) were assayed. The values have been corrected for the values in a control without added protein.

**Fig. 5A.** Western blot of p190/210 and S. pombe FAS total serum; lane 2, 360 ng of p190/210 (fraction VI; lane 3, 360 ng of S. cerevisiae FAS holoenzyme.
compared to reactions where untreated substrate (called "N" for normal) had been used. Fig. 5B summarizes this experiment. As a control, the S. cerevisiae p175SFP1 was used. Johnson and Kolodner (3) showed that the Mg2+ dependence of p175SFP1 in the strand exchange assay can be by-passed using resected linear dsDNA. In this case, joint molecules are formed even in the presence of Ca2+. We confirmed this observation using p140Qoxo2-pretreated linear dsDNA (Fig. 5B; lanes 1 to 3). p140Qoxo2 exhibited low activity in the strand exchange assay (Fig. 5A and Ref. 2). This was also the case when p140Qoxo2 was incubated with pretreated linear duplex in the presence of Ca2+. On the other hand, p190/210 used the p140Qoxo2-pretreated substrate to form joint molecules independent of the present cofactor. There was, in other words, a clear dependence of p190/210 on the exonuclease action of another protein (in this case, p140Qoxo2) acting previously or simultaneously. In order to test whether this requirement was specific for the S. pombe exonuclease, we resected the linear duplex DNA with T7 gene 6 exonuclease, and the strand exchange reaction even in the presence of Ca2+ using dsDNA substrate pretreated with T7 gene 6 exonuclease (Fig. 6).

We wanted to confirm that the joint molecules formed under these conditions fulfilled the structural predictions made. Fig. 11 shows the predicted structures: α-forms, α-forms, and the open circle molecule. Fig. 7 shows a selection of the product molecules seen in such a spread. A numeric evaluation of a spread reaction gave the following numbers: counting 712 linear duplex molecules, we observed 21 α-forms, 11 β-forms, and only 1 open circle. The gap in the open circle molecule (Fig. 7) probably reflects the exonucleolytic degradation of the linear dsDNA substrate. We cannot exclude the possibility that this molecule reflects a broken α-form. These values for the product molecules seem too low when compared to the gel analysis shown in Fig. 6 (lane 6). These values might be an underestimate for two reasons. First, substrate molecules involved in higher order aggregates or joints (we observed 84 such aggregates), consisting of three or more substrate molecules, were not counted in the spreads. Second, the workup for spreading and analysis in the electron microscope might dissociate some structures that were stable enough for gel analysis. In the control reaction, without protein on the same substrate, no joint molecule was seen among 373 linear duplex molecules. In this control reaction, 4 higher order aggregates or joints, as described above, have been observed.

Interaction of p190/210 with DNA—To answer the question, how p190/210 stimulates p140Qoxo2 in the strand exchange reaction and carries out strand exchange on resected substrate, we wanted to know whether and how p190/210 interacts with DNA. The first evidence that p190/210 interacts with DNA came from the purification itself, as both peptides were retained on the ssDNA-cellulose column eluting at 250 mM NaCl. Fig. 8 shows that p190/210 formed stable complexes with ssDNA and dsDNA, as measured by a nitrocellulose filter binding assay. p190/210 bound in a noncooperative way to ssDNA and, to a lower extent, to dsDNA.

Binding of p190/210 to DNA leads to condensation of the DNA into large aggregates which can be sedimented in a table top centrifuge. Fig. 9 shows that 480 ng (2.4 pmol; 80 nM) of p190/210 quantitatively aggregated 0.3 nmol of substrate DNA. This corresponds to about 1 molecule of p190 and p210 per 250 nt of ssDNA or 250 base pairs dsDNA assuming equimolar
S. pombe FAS Mediates DNA Strand Exchange in Vitro—We have purified an activity, termed p190/210, that stimulated the strand exchange activity of p140\textsuperscript{exd} (see accompanying article (2)) and identified it as FAS from S. pombe. The action of both proteins, p140\textsuperscript{exd} and FAS (p190/210), in the strand exchange reaction can be separated. p140\textsuperscript{exd} provides the exonuclease function to produce single-stranded tails on the dsDNA substrate (2, 22). This function can be replaced by T7 gene 6 exonuclease, an exogenous exonuclease arguing against a specific interaction between p140\textsuperscript{exd} and FAS. A number of interactions with DNA were exhibited by FAS. It formed stable protein-DNA complexes with both ssDNA and dsDNA. In addition, FAS condensed DNA to high molecular weight aggregates and was capable of renaturing complementary single-stranded DNA. The structures of the joint molecules produced were confirmed by electron microscopy (Fig. 7). The demonstration of α-forms is of particular importance as it is direct physical evidence for displacement of the homologous strand on the dsDNA by the incoming circular ssDNA.

Role of FAS (p190/210) in the DNA Strand Exchange Reaction—Formation of joint molecules on normal or resected dsDNA substrates is not a spontaneous process under the reaction conditions used as no joint molecules were detected in reactions lacking protein employing gel electrophoresis and electron microscopy for analysis. The effect of FAS was specific shared by many homologous pairing and strand exchange proteins (reviewed in Ref. 1). DNA renaturation was assayed by an S1 nuclease assay in the presence of SDS measuring the production of dsDNA resistant to S1 digestion. Fig. 10 shows that DNA renaturation by p190/210 was a cooperative process. Assuming that DNA aggregation preceded DNA renaturation, the largest portion of the DNA had to be aggregated before extended DNA renaturation could take place (compare Figs. 9 and 10).

DISCUSSION

S. pombe FAS Mediates DNA Strand Exchange on T7 gene 6 exonuclease resected substrate. For the strand exchange reaction, 60 ng (300 fmol; 10 nM) of p190/210 (fraction VI) have been used.

**Fig. 7.** Electron microscopic analysis of p190/210 strand exchange reaction on T7 gene 6 exonuclease resected substrate. For the strand exchange reaction, 60 ng (300 fmol; 10 nM) of p190/210 (fraction VI) have been used.

**Fig. 8.** Formation of protein-DNA complexes by p190/210 with ssDNA (○) and dsDNA (■). Reactions were carried out with the indicated amount of p190/210 (fraction VI). 100% corresponds to 0.3 nmol of substrate DNA. The values were corrected for the nonspecific binding of DNA to the nitrocellulose.

It is important to note that the amount of protein used in the strand exchange assay (1 molecule of p190 and p210 per 100 nt of ssDNA) is able to condense the total substrate DNA into aggregates. Assuming one or more DNA binding sites per monomer (either p190 or p210 or both), the complex structure of the protein in solution (possibly an α6.P6 multitimer as in S. cerevisiae) provides an explanation for the aggregation of DNA by p190/210.

Renaturation of complementary ssDNA is a characteristic representation of the two peptides in the preparation (Fig. 2A).
for the intact protein as heat inactivation eliminated the strand exchange stimulatory activity. We propose the following role of FAS during DNA strand exchange pointing to ways how the rate-limiting step in this reaction can be overcome.

As illustrated in Fig. 11 (I and II), we envision as the first step in this reaction the resection of the dsDNA substrate by a double-stranded DNA exonuclease. This step can be easily separated from the remainder of the reaction, but is essential for the reaction. The next step in the strand exchange reaction (Fig. 11; IV) is the formation of a σ-form molecule, where the circular ssDNA renatures on the tail of the resected duplex. Sikorav and Church (23) proposed DNA condensation (III in Fig. 11), often termed DNA aggregation in the literature (e.g. Ref. 21), as a unifying explanation for the acceleration of DNA renaturation. Three different mechanisms may lead to DNA condensation.

(i) Increased local DNA concentrations can be achieved by macromolecular crowding using inert polymers. Inclusion of 35% dextran sulfate can increase rates of renaturation 100-fold (24). Conditions (37 °C, 15% polyethyleneoxide, 0.3 M NaCl) for spontaneous strand exchange were developed (23). Similar observations were made using 240 base pairs of duplex molecules and M13 size circular ssDNA in the presence of modest concentrations of PEG (≥ 1%).2 We routinely included 6% PEG8000 to enhance sensitivity, but found that the PEG alone did not stimulate the formation of joint molecules under our reaction conditions (see controls in Figs. 1, 5, and 6). This stimulation was attributed to the specific stimulation of the exonuclease activity of p140exo2 on dsDNA (2). In addition, FAS exhibited

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strand exchange activity even in the absence of PEG. Furthermore, the heat inactivation experiment (Fig. 5A) and the relatively low amount (compared to the concentration of crowding reagents) of FAS added to the reaction, argue against a (additional) crowding effect.

(ii) The phenol emulsion reassociation technique (25) is known to accelerate DNA renaturation more than 1000-fold. In the separate consecutive incubation experiment (Fig. 5B) and in the experiments with resected substrate (Fig. 6), the dsDNA was extracted with phenol prior to the assay. Aggregation by phenol could therefore solely involve dsDNA. In the control experiments, no joint molecules could be detected. However, in these reactions (compare Fig. 5B, lanes 1, 8, 10, and 11), an increased amount of label (i.e. duplex DNA) remaining in the slot of the agarose gel is seen. In the electron microscopic analysis on resected substrate, an increased number of entangled substrate molecules (mostly dsDNA) were observed. These aggregates might have been the result of the phenol extraction.

(iii) ssDNA binding proteins such as T4 gene 32 protein (26), SV40 T antigen (27), hnRNP A1 (28), or RecA protein (29) promote DNA renaturation at physiological conditions. Pontius and Berg (30) increased the rate of DNA aggregation by adding cationic detergents observing quantitative as well as qualitative similarities to the DNA aggregation reaction performed by hnRNP A1 (31, 32). Polyvalent cations like spermidine were previously shown to be required for the cationization of DNA rings by topoisomerase due to aggregation of DNA (33). These two examples show that the desired condensation can be attained by neutralizing the DNA with counterions (34). With other ssDNA binding proteins, the direct correlation of DNA condensation and stimulation of strand exchange activity has been demonstrated (7).

The S. cerevisiae FAS is known to form a heterohexamer α6β6 with a M, of 2.4 x 106 (35). Our hydrodynamic data for p190/210 show a large heteromultimer, probably in the same configuration. Assuming one or more DNA binding sites per monomer (either p190 or p210 or both), the hexameric or any multimeric structure would provide an explanation for the DNA aggregation capacity of FAS. Thus, we propose that DNA condensation leads to the α-form molecule shown in Fig. 11 (IV). This structure is stable by the base pairing between the circular ssDNA and the single-stranded tail of the dsDNA (36). Using comparable substrates, the rate-limiting step for a three-strand branch migration has been shown to be annealing (37). We nevertheless see two possible ways that FAS additionally enhances the kinetics in the transition from α-forms (IV in Fig. 11) to the final open circle product (VI in Fig. 11). Similar to a model proposed by Munirappa et al. (37) for the stimulation of E. coli RecA by single-stranded binding, FAS binding to the ssDNA could remove secondary structures in the ssDNA acting as a barrier for branch migration. Alternatively, but not mutually exclusive, FAS could bind to single-strand displaced from the dsDNA; thus, effectively blocking the backwards reaction from the α-form to the α-form. This could be facilitated by the fact that dsDNA is destabilized in the condensed state (discussed in Ref. 23). From studies with RecA it is known that binding to (38) or removal of (39, 40) the displaced strand has an impact on strand exchange.

In Vivo Role of FAS—Fatty acid synthases are cytoplasmic enzymes using acetyl-CoA as a starting block to build saturated long-chain fatty acids using malonyl-CoA as an acyl carrier group. The fatty acid groups are essential for fatty acid synthesis. S. cerevisiae strains carrying mutations in one of the two structural genes for the FAS holoenzyme (FAS1,FAS2) show an absolute requirement for saturated long-chain fatty acids and no meiotic phenotype exhibiting normal spore viability (41). Thus, we cannot rationalize a direct role of FAS in homologous recombination or other DNA metabolic processes. Usually, mutations reducing homologous recombination in S. cerevisiae affect the viability of the meiotic products as homologous recombination and chromosome segregation are functionally intertwined (reviewed in Ref. 42).

Implications for Homologous Pairing and Strand Exchange Proteins—Previous work has revealed possible artifacts in attempts to purify homologous pairing and strand exchange proteins. However, these studies used non-standard reaction conditions (temperature, salt concentration, presence of divalent cations, reaction times, or very short substrates) (23, 36, 43, 44). Previous discussions of the three-strand assay suggested that the demonstration of α-forms (see Fig. 11, V) is indicative of true strand exchange argued by the existence of the displaced strand in α-forms (45). Here, we report strand exchange dependent on an obviously unrelated protein, S. pombe FAS, using normal reaction conditions and substrates, demonstrating strand transfer by electron microscopy (Fig. 7), as indicated by the presence of the displaced strand in α-forms. However, we argue that this activity is not an indication of a role of FAS in DNA metabolism. These findings cast doubt on the specificity of homologous pairing and strand exchange proteins purified with assays using resected substrates (S. cerevisiae DPA, Ref. 46). In addition, the specificity of the assay is compromised when exonuclease activity is present in the reaction as is the case for S. cerevisiae SEP1 (3), S. pombe p140αα (2), D. melanogaster Rpl1 (4), and the human protein HPP-1 (5). In addition, we note a similarity between S. pombe FAS and S. cerevisiae SFI (9, 47) which was also identified as a strand exchange stimulatory factor. By the above arguments we cannot exclude a role in homologous recombination for the aforementioned proteins. However, we want to point out that their biochemical characteristics are not specific to recombination proteins calling for genetic evidence. In fact, for the meiosis-specific DNA strand activity of S. cerevisiae, STPα, a role in meiotic recombination is unlikely by the lack of a spore viability phenotype (48) which is common to all mutants affecting meiotic recombination (42). The data for an involvement of S. cerevisiae SEP1 in homologous recombination are conflicting (49–52). Thus, for systems not easily amenable to genetics, a proof for direct involvement in homologous recombination might be difficult.

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