A Multifunctional Exonuclease from Vegetative Schizosaccharomyces pombe Cells Exhibiting in Vitro Strand Exchange Activity*

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A 140-kDa polypeptide (p140) has been purified over 2000-fold from vegetative Schizosaccharomyces pombe cells using an assay of homologous pairing and strand exchange between linear double-stranded DNA (dsDNA) and circular single-stranded DNA (ssDNA) in vitro. Electron microscopic analysis of the reaction products showed displacement of one strand of the linear duplex DNA by the circular ssDNA molecule. In addition, the protein contained 5' to 3' exonuclease activity on ssDNA and dsDNA (with a 50-fold preference on the single-stranded substrate) as well as on single-stranded RNA. Furthermore, p140 was capable of renaturing complementary ssDNA as shown by SI nuclease assays. p140 behaved like a monomer in solution under reaction conditions. Direct comparison of the biochemical properties, sequence analysis, and cross-reactivity to a monoclonal antibody suggests that p140 is probably identical with ExoII, purified from S. pombe meiotic cells as a ssDNA exonuclease (Szankasi, P., and Smith, G. R. (1992) Biochemistry 31, 6789-6773). Given the diverse activities of p140, the protein might be involved in DNA and/or RNA metabolism in vivo.

Hybrid DNA is supposed to be a central intermediate in homologous recombination (1). After initiation by a single-stranded nick (2) or a double-stranded break (3), invasion of a single-stranded tail into the homologous dsDNA1 partner is postulated, leading to homologous pairing and hybrid DNA formation. The Escherichia coli RecA protein is paradigmatic for proteins that form hybrid DNA (for reviews, see Refs. 4 and 5) and is essential for most but not all homologous recombination in this organism (reviewed in Ref. 6). RecA-independent recombination in E. coli includes plasmid recombination by the RecE pathway (7) and bacteriophage λ recombination by λ exo and β protein (reviewed in Ref. 6). Both pathways have in common exonuclease (8, 9) and DNA renaturation activities (10, 11).

Biochemical assays for hybrid DNA formation developed for RecA have been widely used to purify similar activities from eukaryotic cells using an assay of homologous pairing and strand exchange when exonucleases are present in the reaction. For SEP1, the major mitotic strand exchange activity from Saccharomyces cerevisiae (13), no definite picture about the in vivo role of the protein has emerged yet from genetic analysis (22-25). However, it seems clear that the recombination phenotype cannot be compared to E. coli cells lacking RecA.

Exonucleases play a prominent role at various stages during the recombination process. 5'-3' exonuclease are implicated in the production of 3'-terminal single-stranded tails that are active in homology search and hybrid DNA formation (3). A number of 5'-3' exonucleases from E. coli (9, 26) and bacteriophages (8, 27) are known to be required for recombination (see Ref. 28 for review). Less is known about exonucleases in yeasts and their in vivo functions (29, 30, for review, see Ref. 31).

The fission yeast Schizosaccharomyces pombe is highly diverged from S. cerevisiae (32), but has similar classical and molecular genetics. These characteristics make it a suitable model system for correlating biochemical activities of proteins with biological functions. We have used the strand exchange reaction developed for RecA (33) to purify activities from S. pombe that can form hybrid DNA. In this report we describe the purification and characterization of an S. pombe protein, p140, that catalyzes homologous pairing and strand exchange. In addition, the protein exhibited exonuclease activity on ssDNA and dsDNA as well as on single-stranded RNA. p140 is probably identical with ExoII, purified from S. pombe meiotic cells as a ssDNA exonuclease (30). In the accompanying report (34) we describe the purification and characterization of p190/210 identified as a stimulatory factor for the strand exchange activity of p140. The identification of p190/210 as fatty acid synthase raises doubt on the specificity of the strand exchange reaction when exonucleases are present in the reaction.

EXPERIMENTAL PROCEDURES

Strains—The S. pombe wild type strain 972h* was taken from the strain collection in Bern. The E. coli strain 011' (thyA, deo sup) was the...
host for T7am phage and was obtained from R. Kolodner as RDK 1004.

Assay for RNase and RNase-H Activity—Two different RNA substrates were used to test RNase and RNase-H activity. First, a [α-32P]GTP-labeled RNA M13 DNA hybrid was prepared with E. coli T7 RNA polymerase, using M13 viral DNA as a template (37). The reaction mixture (0.1 ml) contained 1.8 nmol of M13 DNA, UTP, ATP, and CTP (2 nmol each), 62 pmol of [α-32P]GTP (1 x 10^6 cpm/μmol), 1 μM ZnSO4, 0.1 M MgCl2, 0.1 M EDTA, pH 7.9, 10 mM MgCl2, 0.1 mM EDTA, 0.1 mM DTT, 150 mM KCl, 0.5 mM phenylmethylsulfonyl fluoride, and 2 μg of E. coli RNA polymerase. The exonuclease assay was carried out essentially as described (38). The reaction mixture (0.15 ml) contained 0.65 pmol of RNA, heated or not heated prior to incubation with enzyme, 33 mM sodium glycinate buffer, pH 9.5, 2.5 mM MgCl2, 0.6 mg/ml BSA, 25 mM NH4Cl, and 0.5 mM DTT. In the control experiment with E. coli RNA polymerase, RNase-H activity was measured by incubating the HEPE-S KOH, pH 7.9, 20 mM MgCl2, 50 mM MgCl2, and 1 mM DTT. S1 nuclease buffer was 30 mM NaAc, pH 4.5, 1 mM ZnSO4, and 50 mM NaCl. All reactions were incubated for 30 min at 37°C, stopped by adding 0.1 ml of 10% trichloroacetic acid, and further incubated on ice for 10 min. Then the samples were centrifuged for 10 min at 10,000 x g. 4 ml of Ready Safe was added to the pellet of supernatant, and counted in a liquid scintillation counter. Second, an unannealed, uniformly [α-32P]GTP-labeled, 86-nt-long histone H4 in vitro transcript (H4 12/12) was made as described in Ref. 36 with a specific activity of 1 x 10^9 cpm/μmol (11.8 fmol of 3' ends). For the RNase-H assay, 1.7 pmol (20 fmol of 3' ends) of the in vitro transcript was annealed to 5.6 pmol (200 fmol of 3' ends). For the RNase-H assay, 1.7 pmol (20 fmol of 3' ends) of [α-32P]GTP-labeled RNA was annealed to 5.6 pmol (200 fmol of 3' ends) of E. coli RNA polymerase, and the reaction mixture was incubated on ice for 10 min. After addition of 5.4 μl of 10% SDS, the samples were fixed, dried, and exposed to x-ray films (Curix RP1, AGFA).

Strand Exchange Activity—Strand exchange assays were performed as described (13) except that 30 pmol of XbaI-digested, linear M13 DNA and 15 pmol of circular viral M13 DNA were used. The linear duplex DNA was labeled at the 3’ end with [α-32P]GTP using T7 DNA polymerase. The DNA of reactions was analyzed by electrophoresis on 0.8% agarose gels in 40 mM Tris acetate (pH 7.9) and 0.1 mM EDTA. The cell slurry was diluted to 1 μg/ml of buffer containing 10% (v/v) glycerol by chromatography on Mono Q (Pharmacia LKB Biotechnology Inc., HR5/5) at a flow rate of 0.4 ml/min. Active fractions (around 190 mM NaCl and were pooled as fraction IV (14 ml, 20 μg/ml). Conductivity of fraction IV was again adjusted to 150 mM NaCl and loaded on Mono Q (Pharmacia LKB Biotechnology Inc., HR5/5) at a flow rate of 0.5 ml/min, and the column was washed with 5 ml of buffer A/150 mM NaCl. A 12-ml gradient of 150 to 400 mM NaCl in buffer A was applied. 0.5-ml fractions were collected. For strand exchange assay and Western blot, 5 μl of each fraction was assayed, and for single-stranded exonuclease assay 1 μl was used. Active fractions (around 240 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 V (5 ml, 30 μg/ml) which was stored at -20°C.

Other Enzymes—SEP1, referred to here as p175SEP to differentiate it from the proteolytic fragment p132SEP (13) was purified in our laboratory essentially as described in Ref. 19 and kindly provided by A. Holler. Ecoll was kindly provided by P. Szankasi and corresponds to fraction V as described in Ref. 30.

Physical Characterization—The sedimentation coefficient of p140 was determined on 5-20% (w/v) sucrose gradients made as follows: 2.5 ml of 20% (w/v) sucrose solution in 20 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 8 mM MgCl2 were put in a polyallomer tube (Beckman, 13 x 51 mm). Another 2.5 ml of 5% sucrose solution was layered carefully on top of the 20% sucrose solution. The tube was sealed with parafilm, placed horizontally on a cold bench at room temperature for 2 h, and then stored in vertical position at 4°C until use. Linearity of the gradients was confirmed by measuring the refractive index of individual fractions. Fraction V was brought into buffer containing 10% (v/v) glycerol by chromatography on Mono Q (Pharmacia, HR5/5). 100 μl of this protein solution containing 8 μg of p140 were used. Gradients were fractionated (100 μl/fraction), and p140 was assayed by a Western dot-blot. The Stokes radius was determined by gel filtration on Seabead S-300 HR (Pharmacia, 0.78 cm x 30 cm). The column was equilibrated in buffer containing 20 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 8 mM MgCl2, at a flow rate of 20 ml/h. 60 μl of buffer V containing 0.1 mg/ml of protein was applied and analyzed with Western dot-blotting. As markers we used ferritin (66.9 Å, 17.6 S), catalase (52 Å, 13.3 S), aldolase (48.1 Å, 7.3 S), BSA (36.5, 4.3 S), and ovalbumin (4.2 S).

Protein Sequencing—8 μg of p140 (fraction V) were digested with 0.08 μg of trypsin (Sigma) at 37°C. The peptides were separated on a Waters-Millipore model 510 HPLC apparatus using a C8 reversed
RESULTS

Identification and Purification of a Strand Exchange Activity from Mitotic S. pombe Cells—To detect an S. pombe activity that can form hybrid DNA in vitro we used the strand exchange reaction shown in Fig. 1 developed in studies with the E. coli RecA protein (33). Since strand exchange proteins are required in high and stoichiometric amounts, e.g. 1 molecule of RecA is needed for every 3 nt of ssDNA (reviewed in Ref. 4), we increased the sensitivity of the assay by 3'-end-labeling the double-stranded linear substrate. The end-labeling also gave a convenient control for the presence of some activities affecting the structural integrity of the substrate, like phosphatases or 3'→5' exonucleases. In addition, during the course of the experiments we found that 6% PEG₆₀₀₀ enhanced strand exchange activity, and thus 6% PEG₆₀₀₀ was included routinely in the experiments described here. This was based on the observation that inert volume-occupying agents such as polyvinyl alcohol and PEG enhanced RecA in vitro activities like homologous pairing, strand exchange, association with ssDNA, and self-association (42). In addition to assaying fractions for strand exchange activity, we tested them for cross-reactivity with an anti-SEP1 monoclonal antibody that recognized a polypeptide in S. pombe crude extracts. We hypothesized that the cross-

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2 W.-D. Heyer, unpublished result.


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reacting material in S. pombe might represent the homolog to S. cerevisiae SEP1. Since a number of strand exchange activities were reported to have associated exonuclease activity (17, 19, 20), we also monitored exonuclease activity. However, the course of the purification was determined by the activity in the strand exchange assay and not by the antibody cross-reactivity profile or exonuclease assay. The single-stranded exonuclease activity as well as the cross-reacting epitope co-purified on all columns.

Fig. 2 shows the elution profile of a ssDNA-cellulose column and the results of the strand exchange assay for individual column fractions. The mobility of the joint molecules formed was similar to that for the joint molecules formed by S. cerevisiae p17₅SEP₁ used as a positive control (Fig. 2). The partially purified activity of this fraction (fraction III) was further characterized. The activity required the substrates to be homologous, using combinations of M13 and φX174 DNAs (data not shown). In addition, the activity was dependent on Mg²⁺, but did not require a high energy cofactor like ATP (data not shown). Subsequent attempts to purify this activity resulted in a loss of specific activity. We hypothesized that this might be due to a multicomponent system where components are separated during the course of purification. A similar observation, loss of specific activity during purification, has been made for the S. cerevisiae p17₅SEP₁ protein and resulted in the identification and purification of a stimulatory factor (43). To test this hypothesis, we complemented active fractions from a phosphocellulose column eluting around 190 mM NaCl (fraction IV) with the flow-through and neighboring fractions not showing strand exchange activity to identify a hypothetical stimulatory factor. Full strand exchange activity could be restored by complement-
ing fraction IV with a fraction eluting from the phosphocellulose column at 300 mM NaCl (data not shown). This hypothetical stimulatory factor of the strand exchange activity of p140 was purified using the reconstitution assay and is called p190/210 (34). Since this report concentrates on the purification and characterization of p140, we report only the functional interaction of this factor with p140 in the strand exchange reaction (see Fig. 4 and Table II). A detailed report on the purification and characterization of p190/210 is published together with an evaluation of the specificity of the strand exchange reaction in the accompanying paper (34).

p140 was purified to apparent homogeneity and has an apparent molecular weight of 140,000 on SDS-PAGE (Fig. 3). Due to the presence of a stimulatory factor and the inherent difficulty in quantifying the strand exchange assay, we monitored the purification by assaying the associated exonuclease activity (see below). Table I shows the summary of the purification of p140 from vegetative S. pombe cells resulting in a more than 2000-fold purification with a final yield of about 5% of the nuclease activity. For the strand exchange activity of p140 (fraction V), we estimate that about 5% of the label was shifted into the joint molecule band using 100 ng (710 fmol; 23.6 nM) in a standard assay. Under identical reaction conditions, 75 ng of p175SEP (430 fmol; 14 nM) formed about 30% joint molecules (see Fig. 2, lane 2). A similar amount (30%) of activity is reached by the combination of p140 and p190/210 (see also Fig. 2). Therefore, addition of p190/210 resulted in a stimulation of the strand exchange activity of p140 which was confirmed by the electron microscopic analysis of reaction products (see below).

**Formation of α-Form and Open Circle Strand Exchange Products**—The illustration in Fig. 1 of the joint molecules formed during the strand exchange reaction makes very spe-

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**TABLE I**

Purification of p140 from mitotic S. pombe cells (400 g)

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein (mg)</th>
<th>Volume (ml)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg)</th>
<th>Purification (x-fold)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Crude extract</td>
<td>6900</td>
<td>600</td>
<td>1410</td>
<td>0.2</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>II. DEAE-cellulose</td>
<td>4050</td>
<td>900</td>
<td>2550</td>
<td>0.6</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>III. ssDNA-cellulose</td>
<td>24.8</td>
<td>124</td>
<td>350</td>
<td>14.1</td>
<td>24</td>
<td>14</td>
</tr>
<tr>
<td>IV. Phosphocellulose</td>
<td>0.28</td>
<td>14</td>
<td>130</td>
<td>464</td>
<td>2200</td>
<td>5</td>
</tr>
<tr>
<td>V. MonoQ</td>
<td>0.09</td>
<td>3</td>
<td>120</td>
<td>1333</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

* Determined with single-stranded DNA exonuclease assay, see text for explanations.
cific predictions about their structure. Since the joint molecules appear in the gel analysis simply as a band with lower mobility, it was important to confirm the structure of the joint molecules by direct examination in the electron microscope. Reactions with p140 alone, with p140 in combination with p190/210, or as control containing no protein were mounted for electron microscopy as described under "Experimental Procedures." The quantitation of the electron microscopic estimation due to losses of joint molecules during the preparation of the samples for the electron microscope. The stimulation of the samples for the electron microscope was done as described under "Experimental Procedures." Reactions were scored for reaction products (see Fig. 4).

We cannot exclude the possibility that this molecule reflects a broken a-form. The quantitation of the electron microscopic estimation due to losses of joint molecules during the preparation of the samples for the electron microscope. The stimulation of the samples for the electron microscope was done as described under "Experimental Procedures." Reactions were scored for reaction products (see Fig. 4).

**Table II**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Strand exchange assay with P140</th>
<th>Strand exchange assay with P140 + P190/210</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear duplex</td>
<td>323</td>
<td>540</td>
</tr>
<tr>
<td>α-Form</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Open circle</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

Fig. 4. Comparison of the exonuclease activities of p140 and p175SEP in a time course experiment. On ssDNA, 14 ng (100 fmol; 3.3 pmol) of p140 (■) or 87.5 ng (500 fmol; 16.3 pmol) of p175SEP (■) were used. On dsDNA, 66 ng (400 fmol; 13.3 pmol) of p140 (□) or 350 ng (2 pmol; 68 nm) of p175SEP (△) were used.

**Table III**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>p140</th>
<th>p175SEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>Linear ssDNA</td>
<td>Linear dsDNA</td>
</tr>
<tr>
<td>Standard*</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>-Enzyme</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>-MgCl₂</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>-MgCl₂ + CaCl₂ at 5 mM</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Preincubation with NEM*</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>+NaCl at 200 mM</td>
<td>17</td>
<td>10</td>
</tr>
</tbody>
</table>

* 100% for p140 corresponded to 350 pmol of digested nucleotides for ssDNA and 30 pmol for dsDNA. 100% for p175SEP corresponded to 620 pmol for ssDNA and 25 pmol for dsDNA.

p140 and p175SEP were preincubated for 10 min at 30 °C with 10 μM N-ethylmaleimide.

below) to determine which step of the strand exchange reaction was stimulated. DNA renaturation by p140 and p175SEP were not found to be altered by PEG₆₅₀ (not shown). Table IV summarizes the effect of 6% PEG₆₅₀ on the exonuclease activity of p140 and 175SEP. The ssDNA exonuclease activity of p140 remained essentially unchanged, whereas the ssDNA exonuclease activity of 175SEP was found to be inhibited almost 2-fold. In contrast, the activity of the two enzymes on double-stranded substrate was increased 2- and 6-fold, respectively, by the presence of PEG.

p140 Renatures Complementary ssDNA—Renaturation of complementary ssDNA can be viewed as the most reduced homologous pairing reaction. Since p140 exhibited strand exchange activity, we wondered whether it would also be active in this pairing reaction. Therefore, we compared the renaturation capacities of p140 and 175SEP using an assay making use of the ssDNA-specific S1 nuclease (Fig. 6). 200 ng of p175SEP (1.14 pmol; 27 nm) renatures 100 pmol of DNA (25% of the input DNA) within 5 min. This confirmed earlier observations made for the proteolytic fragment of 175SEP (39). In contrast, 200 ng of p140 (1.43 pmol; 47 nm) renatures 44 pmol of DNA (11% of the input DNA) in 5 min (Fig. 7). Therefore, p140 was able to renature complementary ssDNA but less efficiently than 175SEP.

p140 Has RNase Activity—p175SEP has also been purified as XRN1, a 5' → 3' exonuclease activity (38, 44) and RNase-H.
activity, we tested two different substrates in RNase and RNase-H assays. The first substrate was produced with viral M13 DNA put into reaction.

*Fig. 6. DNA renaturation in a protein titration with p175SEP (○) or p140 (●). The degree of renaturation is given as percent of total DNA put into reaction.*

activity (37). Therefore, we tested two different substrates in RNase and RNase-H assays. The first substrate was produced with viral M13 DNA serving as a template for the *E. coli* RNA polymerase as described by Stevens and Maupin (37). The control reactions with *E. coli* RNase-H and RNase-A showed that the substrate was in the expected state, either as RNA-DNA hybrid or as free RNA in the case where it has been heated prior to incubation (data not shown). In addition, the heated substrate was sensitive to the single-stranded specific S1 nuclease, whereas the not heated substrate was resistant to the S1 nuclease (data not shown). While p175SEP exhibited similar RNase-H activity as *E. coli* RNase-H on this substrate (data not shown) as described previously (37), the RNase-H activity of *S. pombe* p140 was only a little over the background and could not be reliably determined (not more than 4 fmol of nt released by 50 fmol of p140 in 30 min in a triplicate experiment). The same substrate was denatured and used to determine RNase activity. The results showed that p140 and p175SEP exhibited comparable RNase activity. While 25 fmol of p140 produced 26.8 ± 12.4 fmol of nt in 30-min reactions (n = 6), 25 fmol of p175SEP produced 37.3 ± 26.3 fmol of nt in 30-min reactions (n = 6). This variance might be in part ascribed to the use of different substrate preparations.

The second substrate was a uniformly labeled, uncapped *in vitro* transcript (see "Experimental Procedures"). There was no detectable loss of label from the substrate incubated with 710 fmol (= 100 ng) of p140 or 570 fmol (=100 ng) of p175SEP as analyzed by gel electrophoresis and autoradiography or measuring trichloroacetic acid-soluble radioactivity (data not shown). The same amount of RNase-A degraded the same substrate completely (data not shown). To monitor RNase-H activity, a 25-nt oligodeoxynucleotide was annealed to the 3' end of the 85-nt pre-mRNA as described under "Experimental Procedures." Whether p140 nor p175SEP showed RNase-H activity on this substrate, whereas the positive control reaction with 0.2 unit (~1 pmol) of RNase-H (Boehringer Mannheim) digested the RNA-DNA hybrid as expected (data not shown).

From these experiments we conclude that p140 exhibits RNase activity like *S. cerevisiae* p175SEP which is considerably lower than its activity on ssDNA (see Table IV). Although some RNase-H activity could be measured, it was so low that we doubt its significance.

*p140 Is the Mitotic Form of ExoII*—In order to be able to clone the gene encoding p140, we attempted amino acid sequencing of the purified protein. We obtained sequence for a tryptic fragment (peak 17) as shown in Fig. 7. Comparison of this amino acid sequence with the deduced amino acid sequence of exo2 showed a perfect match with amino acid 488 to 501 of ExoII (Fig. 7). Exonuclease II was purified by Szankasi and Smith (30) as a single-strand-specific exonuclease from meiotic *S. pombe* cells and is encoded by the *S. pombe* exo2 gene which has been cloned by reverse genetics. The homology to the deduced amino acid sequence for p175SEP in this region is 35% identity and 85% similarity (22–24). Comparison of the entire amino acid sequences of ExoII and p175SEP shows 42% identity and 62% similarity between the two proteins. Therefore, it is likely that *S. pombe* p140 is homologous to p175SEP from *S. cerevisiae*. In addition to the amino acid sequence data, direct comparison of p140 and ExoII from Szankasi and Smith (30) on SDS-polyacrylamide gel electrophoresis and Western blots revealed similar properties of the two proteins, including cross-reaction to an anti-SEP1 monoclonal antibody (Fig. 8). In a Western blot with crude extract from a strain with a disrupted exo2 gene, this cross-reacting material is missing (Fig. 8B). In the p140 preparation from vegetative cells (Fig. 8A, lane 3), an additional band with lower electrophoretic mobility appeared which is absent from the ExoII preparation of Szankasi and

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**Table IV**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>-6% PEG&lt;sub&gt;300&lt;/sub&gt;</th>
<th>+6% PEG&lt;sub&gt;300&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>p140 (100 fmol)</td>
<td>20.4 ± 0.6</td>
<td>16.3 ± 2.6</td>
</tr>
<tr>
<td>ExoII (100 fmol)</td>
<td>19.2 ± 4.2</td>
<td>22.3 ± 3.7</td>
</tr>
<tr>
<td>p175SEP (500 fmol)</td>
<td>73.5 ± 4.9</td>
<td>34.3 ± 7.4</td>
</tr>
</tbody>
</table>

*Fig. 7. Comparison of the NH<sub>2</sub>-terminal amino acid sequence of the peak 17 tryptic fragment of p140 with the deduced amino acid sequence of ExoII<sup>4</sup> and SEP1 (22–24). Homology search and alignment was made using the gap program of the GCG package Version 7.1(33), EMBL/GenBank release 35.0; SwissProt release 25.0.*

*P. Szankasi and G. R. Smith, personal communication.*
S. pombe Strand Exchange Activity

Smith (28) (Fig. 8A, lane 4). We conclude that p140 from mitotic cells is identical with ExoII from meiotic cells described by Szankasi and Smith (30).

p140 Is a Monomer in Solution—To determine the hydrodynamic properties of p140, we estimated its Stokes radius by gel filtration and the sedimentation coefficient by sucrose gradient centrifugation. The experimentally measured values and the calculated parameters are summarized in Fig. 9. The apparent native molecular weight of 148,000 was calculated from a Stokes radius of 35.6 Å and from a sedimentation coefficient of 7.0 S using the procedure of Siegel and Monty (45). The partial specific volume of 0.73256 cm³/g as well as the subunit molecular weight of 152,480 was deduced from the predicted amino acid sequence of the gene. Therefore, p140 was a monomer in solution. The calculated frictional ratio \( f/f_s \) of 1.016 implies an axial ratio of 1 (46) indicating a globular protein.

DISCUSSION

From vegetatively growing S. pombe cells, we have purified to near-homogeneity an activity that can catalyze the formation of hybrid DNA from model substrates using an assay of joint molecule formation between linear duplex and circular single-stranded DNA. This activity, called p140, was stimulated in the strand exchange reaction by another factor called p190/210. In this report we concentrate on p140 and its functional characterization. In the accompanying report (34), we concentrate on p190/210 and identify it as fatty acid synthase from S. pombe. The activity of p190/210 in the strand exchange reaction raised doubts on the specificity of this biochemical assay and is addressed in the accompanying report (34). p140 has been purified using two independent assays, strand exchange and exonuclease. As we were interested in proteins that can form hybrid DNA, the strand exchange assay was taken as the lead. p140 was a monomer in solution under reaction conditions with a native molecular weight of 148,000. It was capable of catalyzing two different pairing reactions: the three-strand reaction between linear duplex and circular single-stranded DNA as shown by gel analysis and electron microscopy as well as the renaturation of complementary ssDNA as shown by S1 nuclease assays. In addition, we discovered that p140 is an exonuclease on ssDNA and dsDNA as well as on single-stranded RNA. The directionality of the exonuclease activity is inferred to be 5' to 3' based on several observa-

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**Fig. 8.** A, comparison of p140 and ExoII. Lanes 1 and 3, 0.4 µg of p140 each; lanes 2 and 4, 0.4 µg of ExoII each. Lanes 1 and 2, Coomassie-stained gel; lanes 3 and 4, Western blot. B, Western blotting of 50 µg of crude extract from a strain with a deletion in the exo2 gene and from a heterozygous strain (exo2+/exo2::ura4+), was sporulated, and two spore clones from a single tetrad (one ura+:exo2-; one ura-:exo2') were analyzed. Lane 1, exo2-; lane 2, exo2+.

**Fig. 9.** Hydrodynamic properties of p140. Stokes radius and sedimentation coefficient of p140 were determined by gel filtration and sucrose gradient centrifugation. Standard curve of the gel filtration (0.78 cm² x 39 cm, Sephacryl S-300 HR) is shown in panel A, left. Standard curve for determination of the sedimentation coefficient is shown in panel A, right. Panel B summarizes the measured and calculated hydrodynamic properties of p140.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stoke's radius (Å)</td>
<td>35.6</td>
</tr>
<tr>
<td>Sedimentation coefficient (x 10⁻¹³ s)</td>
<td>7.0</td>
</tr>
<tr>
<td>Diffusion coefficient (x 10⁷ cm²/s)</td>
<td>6.78</td>
</tr>
<tr>
<td>Predicted subunit molecular weight a</td>
<td>152480</td>
</tr>
<tr>
<td>Native molecular weight</td>
<td>148000</td>
</tr>
<tr>
<td>Perrin shape factor /axial ratio</td>
<td>1.016 / 1</td>
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tions. Firstly, the linear dsDNA substrate in the strand exchange reaction has been end-labeled at the 3’ end but was subject to digestion by p140, whereas, secondly, a 5’-end-labeled molecule was digested (data not shown). Thirdly, as discussed below, p140 is likely to be identical with ExoII purified from meiotic S. pombe cells which was also found to be a 5’ to 3’ exonuclease (30).

Szankasi and Smith (30) have purified a ssDNA-specific exonuclease, ExoII, from meiotic S. pombe cells, an activity apparently unrelated to the assay we used for p140 purification. However, the available evidence suggests that p140 is the mitotic form of ExoII. Both proteins have the same molecular weight determined by SDS-polyacrylamide gel electrophoresis, although in the p140 preparation a slower migrating species is apparent that is absent from the ExoII preparation. This might be due to mitosis-specific post-translational modification of the protein or to proteolysis during the purification. Both proteins cross-react with a monoclonal antibody prepared against the SEP1 protein from S. cerevisiae which is probably the sequence homolog of p140 in that organism. This cross-reacting material highly likely represents the missing in a strain deleted for the ex02 gene. Furthermore, amino acid sequencing of a tryptic peptide from p140 shows a perfect match to the ExoII sequence deduced from the cloned gene.

Some differences exist in the exonuclease activity of p140 and that reported for ExoII (30). The differences in activity on ssDNA substrate between ExoII (reported by Szankasi and Smith (30)) and p140 (Table IV) are probably due to differences in assay conditions or substrate conditions as p140 and ExoII behaved nearly identical on ssDNA substrate in the side-by-side comparison (Table IV). The difference on dsDNA substrate was more pronounced even in the side-by-side comparison and was accentuated by the addition of PEG which specifically stimulated the dsDNA activity (see below). The other activities of p140 reported here including strand exchange activity, exonuclease, and the hydrodynamic properties of the protein cannot be compared as they have not been reported before.

As suggested by the cross-reactivity to the anti-SEP1 monoclonal antibody and the biochemical properties of the two proteins, p140 appears to be the S. pombe homolog to p175SEP of S. cerevisiae. This is strongly corroborated by the sequence data presented in the accompanying report (34) cast doubt on the exonuclease of S. pombe and of p175SEP of S. cerevisiae.

Using an assay related but different to the strand exchange reaction employed here, Arai et al. (18) purified a multicomponent homologous pairing activity from vegetative S. pombe cells consisting of proteins with an M, of 30,000, 65,000, and 100,000. Due to the difference in molecular weight and the reported absence of nuclease activity in the three-protein complex (18), we regard it as different from p140.

The biochemical properties of p140 and can suggest a role in homologous recombination and repair. The exonuclease function is similar to the exonuclease of E. coli (26) which is required for UV resistance and recombination in the RecF pathway (47–49). A similar function could be invoked for p140 and, e.g. the dsDNA exonuclease function could allow a 5’ to 3’ helicase to act from a double-strand break. The liberated 5’-ending strand would be subject to degradation by the more potent ssDNA exonuclease activity. The exposed 3’-ending tail could be used in turn for strand invasion of a homologous DNA duplex by the homologous pairing activity of the protein. Similar arguments would apply to a proposed role of p175SEP from S. cerevisiae (13, 19, 39, 50). However, the phenotypic analysis of mutants in the SEP1 gene has not revealed a strong defect in recombination or repair (25–26). In addition, biochemical data presented in the accompanying report (34) cast doubt on the specificity of the strand exchange reaction used to purify this type of protein. Other roles for p175SEP, e.g. in RNA metabolism, have been considered on the basis of its RNase activity (37, 44, 51). A role in RNA metabolism cannot be excluded for p140. Therefore, further work is needed to determine the in vivo roles of p140 and of p175SEP of S. cerevisiae.

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REFERENCES


S. pombe Strand Exchange Activity

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