*Proc. Natl. Acad. Sci. USA*
Vol. 88, pp. 6780–6784, August 1991
Biochemistry

# *Drosophila* Rrp1 protein: An apurinic endonuclease with homologous recombination activities

(strand transfer/exodeoxyribonuclease/DNA repair)

MIRIAM SANDER*†, KY LOWENHAUPT‡, AND ALEXANDER RICH‡

*Laboratory of Genetics D3-04, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709; and ‡Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139

*Contributed by Alexander Rich, April 26, 1991*

ABSTRACT **A protein previously purified from *Drosophila* embryo extracts by a DNA strand transfer assay, Rrp1 (recombination repair protein 1), has an N-terminal 427-amino acid region unrelated to known proteins, and a 252-amino acid C-terminal region with sequence homology to two DNA repair nucleases, *Escherichia coli* exonuclease III and *Streptococcus pneumoniae* exonuclease A, which are known to be active as apurinic endonucleases and as double-stranded DNA 3′ exonucleases. We demonstrate here that purified Rrp1 has apurinic endonuclease and double-stranded DNA 3′ exonuclease activities and carries out single-stranded DNA renaturation in a $Mg^{2+}$-dependent manner. Strand transfer, 3′ exonuclease, and single-stranded DNA renaturation activities comigrate during column chromatography. The properties of Rrp1 suggest that it could promote homologous recombination at sites of DNA damage.**

The processes of genetic recombination and DNA repair are intensively studied in many laboratories. It has been observed that factors affecting one of these processes can coordinately affect the other. For example, DNA damage responses are associated with elevated recombination rates (1), mutations that cause reduced recombination are associated with UV light or mutagen sensitivity (2–4), and eukaryotic repair proteins are induced during meiosis (5). In some cases, bifunctional roles of specific proteins are known, such as recA protein and recBCD protein, two *Escherichia coli* proteins that are active in both DNA repair and recombination reactions (2, 3).

Eukaryotic proteins that carry out the strand transfer step in homologous recombination have been identified in yeast (6, 7) and other species (8–13). The yeast strand transfer proteins are essential for wild-type recombination levels in either meiotic or mitotic cells (14, 15). In higher eukaryotes, the biological functions of these proteins are unknown.

Apurinic/apyrimidinic (AP) endonucleases are essential for repair of alkylation and oxidative DNA damage. In *E. coli*, one major and several minor AP endonucleases have been characterized (16). Exonuclease III, a class II endonuclease (see ref. 17 for classification of AP endonucleases), represents 85–90% of the activity (18). Under normal growth conditions, endonuclease IV represents 5% of the activity, but oxidative DNA damage can increase its level 10-fold (19).

Analysis of the cDNA sequence of the gene encoding the *Drosophila* strand transfer protein reveals that *Drosophila* recombination repair protein 1 (Rrp1), *E. coli* exonuclease III, and *Streptococcus pneumoniae* exonuclease A form a three-member family of repair endo/exonucleases (unpublished data). A conserved 250-amino acid region is shared among the three proteins. However, *Drosophila* Rrp1 has a 427-amino acid N-terminal region that is not related to proteins in the data base. Enzymatic characterization of the two bacterial enzymes suggests strongly that the homologous domain is sufficient for AP endonuclease and 3′ exonuclease activities (20, 21). The strand transfer activity of Rrp1 has been characterized (13). This activity copurifies with a polypeptide of 105 kDa by electrophoretic mobility during SDS/PAGE. Expression of the gene encoding this polypeptide in *E. coli* has confirmed that it is active in strand transfer (M.S., unpublished results). In this report, we demonstrate that Rrp1 has associated AP endonuclease, 3′ exonuclease, and single-stranded DNA (ssDNA) renaturation activities. This combination of repair and recombination activities has not been observed previously.

## MATERIALS AND METHODS

**Nucleic Acids and Enzymes.** Plasmid DNA substrates were purified by an alkaline lysis procedure (22) followed by chromatography on Qiagen (Qiagen, Studio City, CA). Bacteriophage ssDNA was either purified by Qiagen chromatography (pBluescript ssDNA) or purchased from Pharmacia/LKB (M13 ssDNA and $\phi$X174 ssDNA). Labeled double-stranded DNA (dsDNA) substrates were prepared by standard methods (23). Linear 409-nucleotide (+)-ssDNA and (−)-ssDNA fragments were prepared from the 5′ $^{32}$P-end-labeled 409-base-pair (bp) *Dde* I fragment of pBluescript SK by electrophoresis on a strand separating polyacrylamide gel. Restriction enzymes, exonuclease III, T4 DNA polymerase, and T4 polynucleotide kinase were purchased from New England Biolabs or Life Technologies (Gaithersburg, MD). Bacterial alkaline phosphatase was purchased from Life Technologies. Calf thymus histone H1 was purchased from Sigma. Rrp1 protein was purified as described (13).

**Sequence Analyses.** Nucleotide and protein sequence analyses were carried out using the University of Wisconsin Genetics Computer Group (UWGCG) software package (24). The Rrp1 cDNA nucleotide sequence will be reported separately (unpublished data).§

**AP Endonuclease Assay.** Partially depurinated plasmid DNA was prepared by heat/acid treatment as described (21). The number of AP sites per molecule (0.5) was estimated essentially as described by Kuhnlein *et al.* (25). The fraction of the DNA population lacking apurinic sites was determined by using excess exonuclease III to convert supercoiled DNA substrate to nicked circular DNA, followed by electrophoretic separation of the products and quantitative densitometry. Partially depurinated supercoiled plasmid DNA or untreated plasmid DNA (180 fmol) was preincubated in 20 $\mu$l of a buffer containing 50 mM Tris·HCl (pH 7.5), 50 $\mu$g of bovine

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AP, apurinic/apyrimidinic; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; UWGCG, University of Wisconsin Genetics Computer Group.

†To whom reprint requests should be addressed.

§The sequence for the Rrp1 cDNA has been deposited in the GenBank data base (accession no. M62472).

serum albumin per ml, and either 5 mM $MgCl_2$ or 5 mM $CaCl_2$ for 2 min at 30°C. Reactions were initiated by the addition of enzyme and mixtures were incubated at 30°C. Electrophoresis of reaction products was carried out in Tris acetate/EDTA buffer (23) containing 0.5 μg of ethidium bromide per ml. Photographic negatives of the agarose gels were quantitated by densitometry. Quantitation was carried out on reactions performed in duplicate. Preexisting nicked plasmid (15%) was subtracted from all data points.

An estimation of the specific activity of the Rrp1 AP endonuclease was made by assuming that the major protein species added to the reaction mixture is the active protein and by using the observed initial rate of the endonuclease reaction in the presence of 1.8 fmol of Rrp1 under the assay conditions described. For purposes of comparison to published values, the unit of activity is defined as the amount of protein required to produce a rate of AP site cleavage of 1 pmol·min$^{-1}$. The specific activity of the AP endonuclease activity of exonuclease III was estimated to be $9.1 \times 10^4$ units/mg using data from Levin and Demple (26).

**Analysis of Exonuclease Specificity.** pUC18 plasmid DNA was 5′ $^{32}$P-end-labeled at the *Hin*dIII site and digested with either *Eco*RI or *Ban* II, and the appropriate fragments were isolated by preparative PAGE. The structures of the ends of these fragments are shown at the bottom of Fig. 4. A 322-bp *Pvu* II fragment from pUC18 was 3′ $^{32}$P-end-labeled by an exchange reaction and digested with *Ban* II, and the 120-bp fragment was purified by preparative PAGE. This fragment carries the 3′ end label at a blunt end.

**Chromatography of Rrp1 Protein.** Chromatography on a Mono S HR5/5 column (Pharmacia/LKB) was done essentially as described (13) using buffer S [50 mM Hepes, pH 7.5/25 mM NaCl/0.1 mM EDTA/0.1 mM dithiothreitol/0.25 mM phenylmethylsulfonyl chloride/10% (vol/vol) glycerol] adjusted with NaCl to the appropriate ionic strength. Chromatography on ssDNA agarose (Life Technologies) was carried out in buffer S as described (13).

**Strand Transfer, Exonuclease, and ssDNA Renaturation Assays.** Strand transfer activity was assayed by the method of McCarthy *et al.* (27). Reactions were carried out in 10-μl volumes containing 20 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol, 50 μg of bovine serum albumin per ml, 6 ng of pBluescript (−)-ssDNA, and ≈0.25 ng of a homologous dsDNA fragment 5′ $^{32}$P-end-labeled on the (+)-strand. Reactions were initiated by addition of enzyme, incubated for 10 min at 37°C, and terminated by addition of SDS, EDTA, and proteinase K to concentrations of 0.5%, 12 mM, and 15 μg/ml, respectively. After incubation at 45°C for 10 min, samples were analyzed by agarose gel electrophoresis and quantitated as described (13). The 3′ exonuclease activity was assayed by measuring loss of a 3′ end-specific $^{32}$P label incorporated as the last nucleotide of a blunt-ended dsDNA fragment. The reaction was carried out under strand transfer conditions and included nonhomologous ssDNA to facilitate comparison of exonuclease and strand transfer assays. Reaction products were resolved by agarose gel electrophoresis and duplicate samples were quantitated as described, with the exception that values were not normalized to the total counts per lane. ssDNA renaturation activity was measured under strand transfer assay conditions. The ssDNA substrates for renaturation were a 5′-$^{32}$P-end-labeled 409-nucleotide (+)-ssDNA fragment and homologous circular 2964-nucleotide pBluescript phage (−)-ssDNA. Samples were analyzed by agarose gel electrophoresis and quantitated as described (13). Background spontaneous renaturation was ≤2% (Table 1).

Table 1. Properties of the Rrp1 ssDNA renaturation activity

| DNA substrates, ss linear/ ss circular | Enzyme, ng | NaCl, mM | $MgCl_2$, mM | % product |
|---|---|---|---|---|
| +/− | — | — | 10 | 2 (±1)* |
| +/− | Rrp1, 30 | — | 10 | 89 (±2)* |
| −/− | Rrpl, 30 | — | 10 | 1 |
| +/− | Rrpl, 30 | — | — | 1 |
| +/− | Rrpl, 30 | 50 | 10 | 84 (±2)* |
| +/− | Rrpl, 30 | 100 | 10 | 70 (±11)* |
| +/− | Rrpl, 30 | 200 | 10 | 5 (±1)* |
| +/− | Histone H1, 20 | — | 10 | 93 (±2)† |
| +/− | Histone H1, 20 | 100 | 10 | 91 (±2)† |
| +/− | Histone H1, 20 | 200 | 10 | 90 (±1)† |
| +/− | Histone H1, 20 | — | — | 90 (±2)† |
| +/− | Histone H1, 20 | 100 | — | 88 (±1)† |
| +/− | Histone H1, 20 | 200 | — | 86 (±1)† |

Complete reaction mixtures contain 4 ng of pBluescript phage (−)-ssDNA and a (+)-strand linear 409-nucleotide fragment. As a control reaction, a (−)-strand linear 409-nucleotide fragment was included and the (+)-strand linear fragment was omitted. All incubations were for 10 min at 37°C. Values in parentheses are SD.
*Values determined in triplicate.
†Values determined in duplicate.

## RESULTS

The homology relationships among the three proteins, *Drosophila* Rrp1, *E. coli* exonuclease III, and *S. pneumoniae* exonuclease A, are presented schematically in Fig. 1. A conserved 250-amino acid region is shared among the three proteins. Within this region, there is a 40% or a 33% identity between the *Drosophila* protein and exonuclease A or exonuclease III, respectively. However, *Drosophila* Rrp1 has an N-terminal region of 427 amino acids that is not related to proteins in the data base. A consensus sequence for this protein family indicating all conserved residues is shown in Fig. 2. Six regions >10 amino acids long with ≥58% identity among all three proteins are underlined and denoted by roman numerals. This high level of conservation suggests that the function of this domain is likely to have been preserved.

The enzymatic properties of the two bacterial enzymes suggests strongly that the homologous domain is sufficient for AP endonuclease and 3′-exonuclease activities (20, 21). However, exonuclease III is not active in strand transfer or ssDNA renaturation assays under conditions in which Rrp1 is active (M.S., unpublished results). Although the function of the N-terminal region of Rrp1 is not yet defined, the strand transfer and ssDNA renaturation activities of this protein are likely to depend on the presence of this region.

To confirm the prediction that Rrp1 is enzymatically similar to the bacterial endo/exonucleases, we assayed for AP endonuclease activity. Supercoiled plasmid DNA was partially depurinated by heat/acid treatment to produce 0.5 AP site per molecule. In the presence of Rrp1, supercoiled depurinated plasmid is converted to the nicked circular form (Fig. 3*A*, lanes 2 and 5). Untreated plasmid DNA is not nicked, even in the presence of 500-fold more Rrp1 than

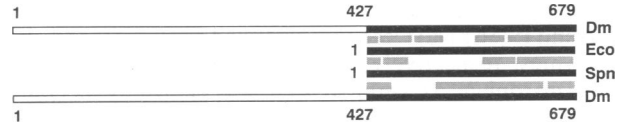


FIG. 1. Homology relationships between Rrp1, exonuclease III, and exonuclease A. Pairwise comparisons of the three protein sequences were made using the COMPARE program of UWGCG (24) and adapted for a schematic diagram. Open boxes represent the unique region of Rrp1; solid boxes represent the homologous region of Rrp1 (Dm), exonuclease III (Eco), and exonuclease A (Spn); stippled boxes between adjacent solid sequence boxes are regions where identity between the pair of sequences is ≥50%. Amino acid sequence coordinates are given.


FIG. 2. Alignment of the amino acid sequences of Rrp1, *E. coli* exonuclease III, and *S. pneumoniae* exonuclease A. Each of the three protein sequences were aligned pairwise using the BESTFIT program from the UWGCG sequence analysis software (24). Alignment of the three sequences together was then performed by visual inspection. Coordinates of each sequence are indicated at the left (Dm, Rrp1; Spn, exonuclease A; Eco, exonuclease III). Boxes surround all residues in common between two or three of the sequences. The consensus sequence indicates residues in common between two sequences in lowercase letters and residues in common between three sequences in capital letters. Regions of high homology are underlined and indicated by roman numerals.

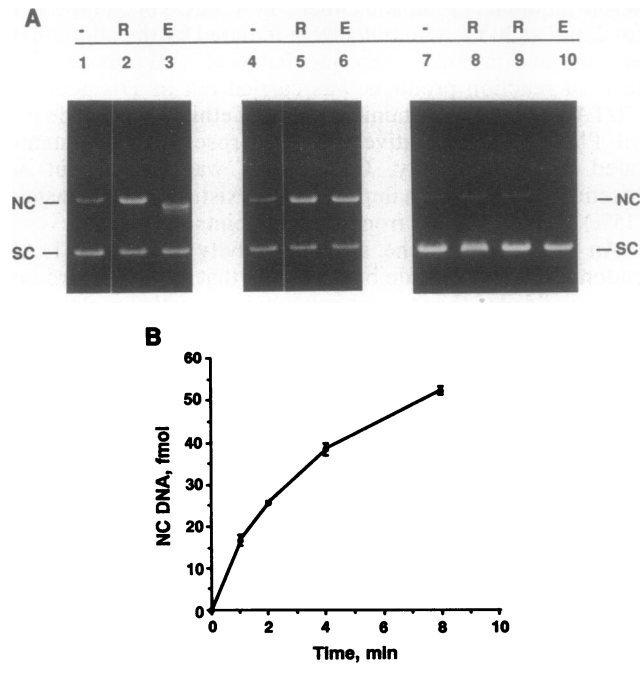


FIG. 3. Specificity of Rrp1 endonuclease activity for depurinated plasmid DNA. (*A*) Partially depurinated supercoiled plasmid DNA (lanes 1–6) or untreated plasmid DNA (lanes 7–10) (180 fmol) was preincubated in 20 $\mu$l of a buffer containing 50 mM Tris·HCl (pH 7.5), 50 $\mu$g of bovine serum albumin per ml, and either 5 mM $MgCl_2$ (lanes 1–3 and 7–10) or 5 mM $CaCl_2$ (lanes 4–6) for 2 min at 30°C. Reactions were initiated by the addition of 90 fmol of Rrp1 (lanes 2, 5, and 9), 900 fmol of Rrp1 (lane 8), 140 fmol of exonuclease III (lanes 3, 6, and 10), or no enzyme (lanes 1, 4, and 7) and incubated for 10 min at 30°C. Reaction products were analyzed by agarose gel electrophoresis. R, Rrp1; E, exonuclease III; SC, supercoiled; NC, nicked circular. (*B*) Depurinated plasmid DNA (180 fmol) was incubated with 1.8 fmol of Rrp1 at 30°C for the indicated period of time. Buffer conditions were the same as in *A*. Average values of duplicate samples are shown.

required to cleave the AP sites (compare Fig. 3*A*, lane 8, and Fig. 3*B*). The limit conversion of the AP DNA substrate to nicked circular DNA is the same for Rrp1 and exonuclease III (compare lanes 5 and 6). The nicked circular product is a poor substrate for the Rrp1-associated 3′ exonuclease (lane 2). In contrast, at similar stoichiometries with respect to AP sites, exonuclease III efficiently degrades nicked circular DNA (lane 3). The Rrp1-associated AP endonuclease requires $Mg^{2+}$ or $Ca^{2+}$ and has no uracil *N*-glycosylase activity (data not shown). In the presence of 1.8 fmol of Rrp1, the initial rate of the nicking reaction is >10 fmol·min$^{-1}$, indicating that enzyme turnover is observed (Fig. 3*B*). This reaction rate slows as product accumulates, producing a nonlinear curve.

AP endonucleases are divided into four classes based on the structure of the 5′ and 3′ termini produced by phosphodiester backbone cleavage (17). Based on its relationship to exonuclease III, it is likely that Rrp1 is a class II AP endonuclease. This characteristic, as well as chromatographic properties and protein electrophoretic mobility, differentiate Rrp1 from the three previously identified *Drosophila* AP endonucleases. *Drosophila* AP endonucleases I (63 kDa) and II (66 kDa) were characterized as class III and class I enzymes, respectively, by Spiering and Deutsch (28). A third *Drosophila* AP endonuclease was cloned by its homology to an AP endonuclease from human cells and has a predicted molecular mass of 34 kDa (29). Since only class II AP endonucleases can remove blocking groups from the 3′ ends of DNA to produce active primers for DNA repair synthesis by polymerases (30, 31), it has been argued that at least one class II AP endonuclease is essential in DNA repair reactions involving AP sites.

In earlier work we reported a 3′ exonuclease activity associated with the strand transfer protein. This result is consistent with the fact that the bacterial nucleases to which Rrp1 is related have a similar activity. Fig. 4 demonstrates the substrate specificity of this 3′ exonuclease. dsDNA with a unique 5′ $^{32}P$ label at a 4-nucleotide 5′ protruding end and either a 4-nucleotide 3′ recessed end on the labeled strand (Fig. 4, lanes 1–5; 51-bp substrate) or a 4-nucleotide 3′ protruding end on the labeled strand (lanes 6–10; 49-bp substrate) was incubated with Rrp1 and $Mg^{2+}$. Up to 17 nucleotides are removed from the 51-bp substrate (lanes 2–5). By contrast, the 49-bp substrate is a relatively poor substrate (lanes 7–10), indicating that protruding 3′ ends and protruding 5′ ends are poorly recognized by Rrp1. As a control, a 120-bp dsDNA fragment 3′ $^{32}P$-labeled at a unique *Pvu* II end was included in each reaction mixture. The 3′ end label was removed from this blunt-ended substrate by Rrp1 in all incubations (lanes 2–5 and 7–10). In the absence of $Mg^{2+}$ no exonuclease activity was observed (data not shown).

Since 5′ protruding and 3′ protruding DNA termini of dsDNA (Fig. 4) as well as the termini of ssDNA linear fragments (data not shown) are all relatively poor substrates for the Rrp1 exonuclease, the preferred substrate of the activity is a base-paired 3′ terminus. The difference in rate of degradation of dsDNA and ssDNA 3′ ends was estimated to be ≈8-fold (data not shown). This result is consistent with the observation that displaced ssDNA is observed during the strand transfer reaction (13). It is possible that limited exonucleolytic degradation of dsDNA 3′ ends is important in the initiation of a three-strand recombination reaction.

We tested whether the exonuclease and strand transfer activities of Rrp1 are tightly coupled by asking if the exonuclease was active under conditions that do not support strand

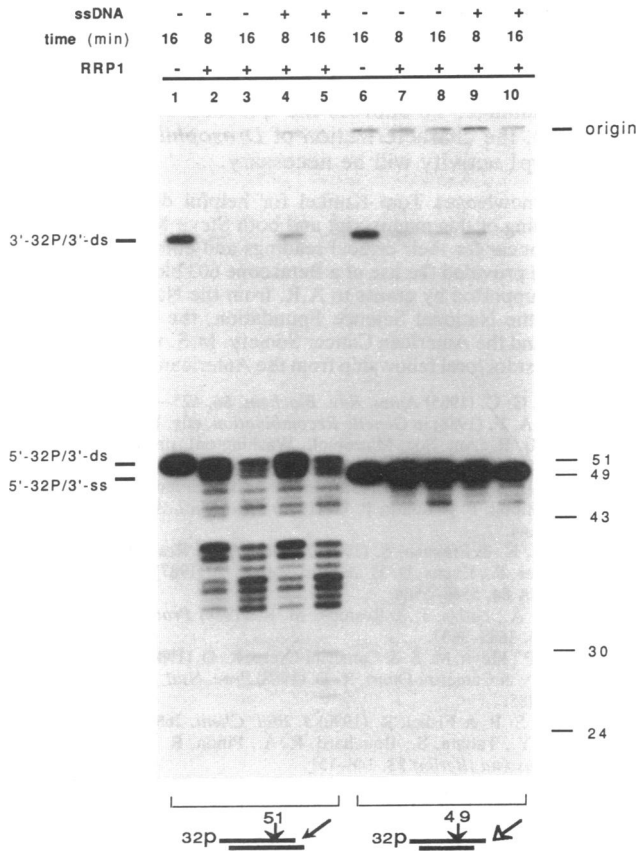


FIG. 4. Specificity of Rrp1 exonuclease for 3′ recessed DNA termini. dsDNA fragments were added to reaction mixtures as follows: lanes 1–5, a 51-bp fragment with a unique 5′ $^{32}$P end label and a 4-nucleotide 3′ recessed end on the labeled strand (5′-$^{32}$P/3′-ds); lanes 6–10, a 49-bp fragment with a unique 5′ $^{32}$P end label and a 4-nucleotide 3′ protruding end on the labeled strand (5′-$^{32}$P/3′-ss); lanes 1–10, a 120-bp fragment with a unique 3′ $^{32}$P-labeled blunt end (3′-$^{32}$P/3′-ds). The 5′ $^{32}$P-end-labeled substrates are diagrammed at the bottom of the figure: solid diagonal arrow indicates the sensitive 3′ end, open diagonal arrow indicates the insensitive 3′ end. Additions of Rrp1 (30 ng) or $\phi$X174 ssDNA (4 ng) and incubation times are indicated. Reaction volumes were 10 $\mu$l. Samples were analyzed by denaturing PAGE followed by autoradiography of the dried gel. The mobility of marker fragments (bp) is shown.

transfer. Fig. 4 shows that the exonuclease is active in both the presence and the absence of nonhomologous ssDNA and therefore is not tightly coupled to strand transfer. A small inhibition is observed if ssDNA is included in the reaction (compare the 120-bp fragment; lanes 2 and 4). However, since the kinetics of the strand transfer and exonuclease reactions are similar (data not shown), it is possible that processing of the 3′ end usually occurs before a strand displacement reaction is initiated.

We have previously demonstrated the coelution of Rrp1 protein with strand transfer activity during column chromatography (13). To test the assertion that AP endonuclease, 3′ exonuclease, and strand transferase activities are properties of a single protein, we determined the elution profiles of the 3′ exonuclease and strand transferase during chromatography on Mono S and ssDNA agarose columns. Fig. 5 shows that the two activity peaks comigrate during both types of chromatography. Coelution of the exonuclease and strand transfer activities was also observed when the peak from the ssDNA agarose column was applied to a Superose 6 gel filtration column (data not shown). In all cases, the elution positions of the activities and Rrp1 protein correspond (13). Titrations of the peak fractions from the Mono S and ssDNA

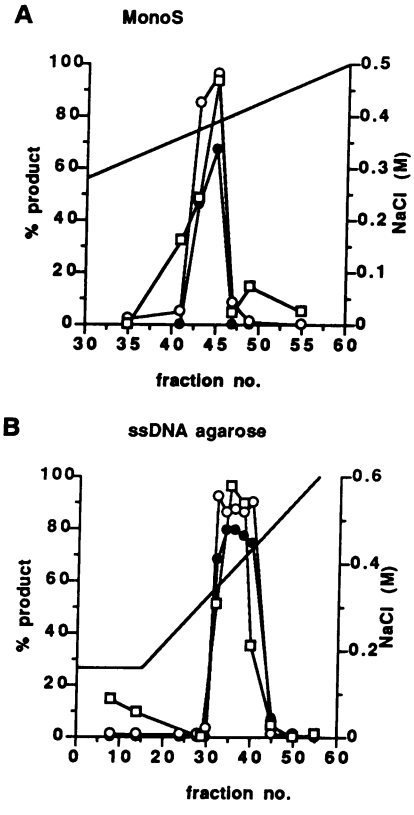


FIG. 5. Coelution of Rrp1 activities during Mono S and ssDNA agarose chromatography. Rrp1 was partially purified by Bio-Rex70 and Superose 6 chromatography as described (13). (*A*) Approximately 1 mg of protein was applied to a Mono S column and fractions were assayed for strand transfer activity (●), ssDNA renaturation (○), and exonuclease activity (□). (*B*) The peak fractions from the Mono S column were pooled, applied to a ssDNA agarose column (13), and analyzed as described in *A*.

agarose columns demonstrate similar ratios of the two activities (data not shown), providing additional support for the hypothesis that Rrp1 is responsible for both the 3′ exonuclease and strand transfer activities.

The strand transfer reaction promoted by Rrp1 implies that the protein interacts with ssDNA and can promote homologous pairing of DNA strands. These properties are also demonstrated by a ssDNA renaturation assay. Table 1 shows that Rrp1 efficiently renatures homologous ssDNA. In addition, the elution profile of ssDNA renaturation activity comigrates with Rrp1 protein during Mono S and ssDNA agarose chromatography (Fig. 5). Renaturation of ssDNA by strand transfer proteins (32–34) and histone H1 (35) has been described by others. The renaturation activity of Rrp1 is clearly distinguished from histone H1-promoted ssDNA renaturation by its dependence on $Mg^{2+}$ and its sensitivity to inhibition by NaCl (Table 1). Since exonuclease III has no detectable ssDNA renaturation activity in this assay (data not shown), ssDNA renaturation by Rrp1 may require a function provided by its N-terminal region.

## DISCUSSION

A relationship and coordination between DNA repair and homologous recombination processes has been observed in several organisms including *Drosophila* (4), *E. coli* (2, 3), and yeast (5). The data presented above suggest that Rrp1 could be important in both DNA repair and homologous recombination events, since we have demonstrated that both homologous recombination and DNA repair activities are present in a protein fraction that is >85% homogeneous for a single

protein (13). It is likely that the demonstrated activities are the properties of the major polypeptide present for the reasons discussed below.

In the presence of 1.8 fmol of Rrp1, the initial rate of nicking of AP DNA is >10 fmol·min$^{-1}$ (Fig. 3*B*), indicating an enzyme specific activity of $\approx 10^5$ units/mg, which is comparable to the specific activity of $2 \times 10^5$ units/mg reported for HeLa cell AP endonuclease (36). Since the fraction assayed is >85% homogenous, this suggests that Rrp1 protein is responsible for the observed AP endonuclease activity.

The homologous region shared by Rrp1, exonuclease III, and exonuclease A is likely to be sufficient for AP endonuclease and 3′ exonuclease activities. Our data are consistent with this, since the exonuclease activity present in the Rrp1 fraction has a specificity identical to that of the bacterial enzymes (Fig. 4). This exonuclease coelutes with strand transfer activity and the Rrp1 protein during column chromatography (Fig. 5 and ref. 13). In addition, the ratio of the strand transfer activity to the exonuclease activity does not change significantly during purification by ssDNA agarose chromatography (data not shown).

We have previously demonstrated that the Rrp1-catalyzed strand transfer reaction proceeds by a strand displacement mechanism (13). Thus, it is unlikely that an artifactual strand transfer activity is observed resulting from degradation of dsDNA followed by renaturation of homologous ssDNA. The observation that a $Mg^{2+}$-dependent and salt-sensitive ssDNA renaturation activity copurifies with the strand transfer activity and the 3′ exonuclease activity (Fig. 5 and Table 1) is consistent with this interpretation.

The strand transfer and ssDNA renaturation reactions require an interaction of the active protein with ssDNA. Several related characteristics of the Rrp1 nuclease activities can be explained by the presence of a ssDNA binding domain in the Rrp1 protein. The inability of Rrp1 exonuclease to act efficiently at a nick in circular dsDNA could reflect relatively tight binding to a short single-stranded region in the double-stranded plasmid. The same property would cause product inhibition of the AP endonuclease and result in a limited linear range of that reaction. The limited 3′ exonucleolytic degradation of dsDNA (Fig. 4) could be due to interaction with the 5′ single-stranded tail on the DNA substrate.

Thus, we suggest that Rrp1 protein may be responsible for the homologous recombination and DNA repair activities observed. However, further physical and enzymatic characterization will be required to establish structure–function relationships for this protein. The 679-amino acid protein, synthesized in *E. coli* from an expression vector carrying the Rrp1 cDNA, has an electrophoretic mobility identical to that of the native *Drosophila* protein and is capable of promoting ATP-independent strand transfer both in the crude *E. coli* extract and after partial purification (M.S., unpublished results). This suggests that the Rrp1 polypeptide is sufficient for the strand transfer function. A detailed structure–function analysis can now be carried out by the expression of deleted or altered variants of the protein. A mechanistic analysis of the strand transfer reaction will also be of interest.

The structure and enzymatic properties of Rrp1 suggest that it may be important for DNA repair in *Drosophila*. In *E. coli*, exonuclease III is the major AP endonuclease. Mutants in the gene for exonuclease III (*xth*) are slightly sensitive to ionizing radiation, oxidative damage, and alkylating agents (37, 38) and are inviable when in a *dut* background, which causes increased uracil incorporation into DNA (39, 40). Therefore, a major *in vivo* function of exonuclease III involves recognition of sites of DNA damage and preparation of the damaged DNA for repair. An *in vivo* role of Rrp1 may be to facilitate recombinational repair of DNA damage at or adjacent to abasic sites. The recombination activities associated with Rrp1 may also be important in promoting homologous recombination events that are not initiated or induced by DNA damage. To address the question of its biological function(s), the characterization of *Drosophila* strains deficient in Rrp1 activity will be necessary.

M.S. acknowledges Tom Kunkel for helpful discussion and a careful reading of this manuscript and both Steve Kowalczykowski and Aziz Sancar for their critical readings and comments. Betagen Corporation provided the use of a Betascope 603 blot analyzer. This work was supported by grants to A.R. from the National Institutes of Health, the National Science Foundation, the Office of Naval Research, and the American Cancer Society. M.S. was supported in part by a postdoctoral fellowship from the American Cancer Society.

1. Walker, G. C. (1985) *Annu. Rev. Biochem.* **54**, 425–457.
2. Taylor, A. F. (1988) in *Genetic Recombination*, eds. Kucherlapati, R. & Smith, G. R. (Am. Soc. Microbiol., Washington), pp. 231–263.
3. Smith, G. R. (1987) *Annu. Rev. Genet.* **21**, 179–201.
4. Baker, B. S., Boyd, J. B., Carpenter, A. T., Green, M. M., Nguyen, T. D., Ripoll, P. & Smith, P. D. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4140–4144.
5. Madura, K. & Prakash, S. (1990) *Nucleic Acids Res.* **18**, 4737–4742.
6. Kolodner, R., Evans, D. H. & Morrison, P. T. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5560–5564.
7. Sugino, A., Nitiss, J. & Resnick, M. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3683–3687.
8. Hsieh, P., Meyn, M. S. & Camerini-Otero, R. D. (1986) *Cell* **44**, 885–894.
9. Eisen, A. & Camerini-Otero, R. D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7481–7485.
10. Moore, S. P. & Fishel, R. (1990) *J. Biol. Chem.* **265**, 11108–11117.
11. Hotta, Y., Tabata, S., Bouchard, R. A., Pinon, R. & Stern, H. (1985) *Chromosoma (Berlin)* **93**, 140–151.
12. Ganea, D., Moore, P., Chekuri, L. & Kucherlapati, R. (1987) *Mol. Cell. Biol.* **7**, 3124–3130.
13. Lowenhaupt, K., Sander, M., Hauser, C. & Rich, A. (1989) *J. Biol. Chem.* **264**, 20568–20575.
14. Dykstra, C. C., Kitada, K., Clark, A. B., Hamatake, R. K. & Sugino, A. (1991) *Mol. Cell. Biol.* **11**, 2576–2582.
15. Clark, A. B., Dykstra, C. C. & Sugino, A. (1991) *Mol. Cell. Biol.* **11**, 2583–2592.
16. Sancar, A. & Sancar, G. B. (1988) *Annu. Rev. Biochem.* **57**, 29–67.
17. Linn, S., Demple, B., Mosbaugh, D. W., Warner, H. R. & Deutsch, W. A. (1981) in *Chromosome Damage and Repair*, eds. Seeberg, E. & Kleppe, K. (Plenum, New York), pp. 97–112.
18. Levin, J. D., Johnson, A. W. & Demple, B. (1988) *J. Biol. Chem.* **263**, 8066–8071.
19. Chan, E. & Weiss, B. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3189–3193.
20. Rogers, S. G. & Weiss, B. (1980) *Methods Enzymol.* **65**, 201–211.
21. Puyet, A., Greenberg, B. & Lacks, S. A. (1989) *J. Bacteriol.* **171**, 2278–2286.
22. Birnboim, H. C. (1983) *Methods Enzymol.* **100**, 243–255.
23. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), Vol. 2.
24. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387–395.
25. Kuhnlein, U., Penhoet, E. E. & Linn, S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1169–1173.
26. Levin, J. D. & Demple, B. (1990) *Nucleic Acids Res.* **18**, 5069–5075.
27. McCarthy, J. M., Sander, M., Lowenhaupt, K. & Rich, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5854–5858.
28. Spiering, A. L. & Deutsch, W. A. (1986) *J. Biol. Chem.* **261**, 3222–3228.
29. Kelley, M. R., Venugopal, S., Harless, J. & Deutsch, W. A. (1989) *Mol. Cell. Biol.* **9**, 965–973.
30. Warner, H. R., Demple, B. F., Deutsch, W. A., Kane, C. M. & Linn, S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4602–4606.
31. Franklin, W. A. & Lindahl, T. (1988) *EMBO J.* **7**, 3617–3622.
32. Heyer, W. D., Evans, D. H. & Kolodner, R. D. (1988) *J. Biol. Chem.* **263**, 15189–15193.
33. McEntee, K. (1985) *Biochemistry* **24**, 4345–4351.
34. Bryant, F. R. & Lehman, I. R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 297–301.
35. Cox, M. M. & Lehman, I. R. (1981) *Nucleic Acids Res.* **9**, 389–400.
36. Kane, C. M. & Linn, S. (1981) *J. Biol. Chem.* **256**, 3405–3414.
37. Demple, B., Halbrook, J. & Linn, S. (1983) *J. Bacteriol.* **153**, 1079–1082.
38. Sammartino, L. J. & Tuveson, R. W. (1983) *J. Bacteriol.* **156**, 904–906.
39. El-Hajj, H. H., Zhang, H. & Weiss, B. (1988) *J. Bacteriol.* **170**, 1069–1075.
40. Taylor, A. F. & Weiss, B. (1982) *J. Bacteriol.* **151**, 351–357.