Ultraviolet Damage Endonuclease (Uve1p): A Structure and Strand-Specific DNA Endonuclease†

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ABSTRACT: *Schizosaccharomyces pombe* ultraviolet damage endonuclease (UVDE or Uve1p) performs the initial step in an alternative excision repair pathway for UV-induced DNA damage. This DNA repair pathway was originally thought to be specific for UV damage. However, the broad substrate specificity of Uve1p suggests a more general role for this enzyme. Uve1p recognizes UV-induced bipyrimidine photoadducts and other non-UV-induced DNA adducts. Biochemical and genetic analysis also suggests that Uve1p may be involved in orchestrating mismatch repair in vivo. This study demonstrates that Uve1p recognizes and cleaves heteroduplex DNA with small unpaired loops but does not recognize loops six to eight nucleotides in length. In addition, the enzyme does not recognize DNA with palindromic insertions that could form base-paired hairpin structures. The cleavage efficiency of Uve1p depends on the distance of a mismatch from the DNA terminus, suggesting that the 3′ terminus may contribute to the strand discrimination signal for Uve1p. These biochemical activities are discussed in the context of the role of Uve1p in DNA repair.

The integrity of its genetic material must be maintained for a biological species to survive. However, DNA is continuously subject to damage by endogenous and exogenous agents that can lead to mutations, neoplasia, or cell death (*1*, *2*). One potential source of mutations is nucleotide misincorporation by DNA polymerases during DNA replication or repair. In addition, primer/template slippage can occur at repetitive DNA sequences during replication, resulting in single-stranded loops of one or more unpaired bases called insertion/deletion loops (IDLs)[1] that can be mutagenic (*3*). The human genome has an abundance of simple repeat sequences that are relatively unstable (*4*). Expansion of such repeat sequences has been associated with human genetic diseases, including Huntington's disease, fragile X syndrome, and myotonic dystrophy (*5*).

The *Escherichia coli* Mut HLS pathway has been extensively characterized and is the prototypical DNA mismatch repair (MMR) pathway. This repair pathway recognizes and repairs small IDLs and all single-base mismatches except C•C in a strand-specific manner (*6*). Mismatch repair pathways have been highly conserved during evolution (*7*). Eukaryotes, including *Saccharomyces cerevisiae*, and humans have several genes encoding proteins homologous to bacterial MutL and MutS (*3*). For example, there are six MutS (*MSH1*−*6*) and four MutL (*MLH1*−*3* and *PMS1*) homologues in *S. cerevisiae* (*8*). The Msh2p−Msh6p heterodimer binds base mismatches and small IDLs, whereas the Msh2p−Msh3p heterodimer binds only small and large IDLs (*9*). A considerable amount of evidence implicates mismatch repair in stabilizing repetitive DNA sequences (*9*−*11*).

UV damage endonuclease (Uve1p) is a DNA repair enzyme from *Schizosaccharomyces pombe* that was identified on the basis of its ability to complement the repair defect in *uvrA recA phr E. coli* (*12*). Uve1p initiates the removal of UV photoproducts via the alternative excision repair pathway (AER) by incising the DNA phosphodiester backbone immediately 5′ to the site of a bipyrimidine photoproduct (*12*−*15*). Homologues of Uve1p exist in *Neurosporra crassa* and *Bacillus subtilis* (*12*, *16*). The gene for Uve1p has been identified, and its sequence encodes a 68 kDa protein (*17*).

Overexpressed, purified full-length Uve1p is unstable (*13*, *18*). For the majority of our previous biochemical studies, we have utilized a glutathione *S*-transferase fusion with a fragment of Uve1p lacking its N-terminal 228 amino acids (*13*, *18*, *19*). Enzymatic studies reveal that UV-induced DNA photoproducts and other bulky and nonbulky DNA lesions are substrates for Uve1p (*19*, *20*). In addition, Uve1p recognizes mispaired bases in duplex DNA and cleaves the phosphodiester bonds immediately, one and two nucleotides (nt) 5′ to the mispaired base in an ATP-independent manner (*18*). Mutants lacking Uve1p display a mutator phenotype,

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[1] Abbreviations: AER, alternative excision repair; GΔ228Uve1p, GST-tagged truncated version of Uve1p; IDL, insertion deletion loop; MMR, mismatch repair; nt, nucleotide; Uve1p, gene product of the Uve1 gene (ultraviolet damage endonuclease).

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which confirms the notion that Uve1p plays a role in MMR (*18*). Biochemical and genetic evidence has implicated Rad2p in steps after Uve1p in the AER pathway (*21*−*23*). *rad2*Δ mutants of *S. pombe* also have an increased rate of duplication of sequences flanked by short repeats (*24*). These results indicate that the AER components in *S. pombe* also contribute to MMR.

Genetic analysis of meiotic recombination events indicates that there are at least two pathways for MMR in *S. pombe* (*25*, *26*). A major, long-patch MMR system which recognizes all mismatch combinations except C·C mispairs is thought to be a counterpart to the *E. coli* MutHLS pathay. *S. pombe* homologues of MutL and MutS have been identified by sequence homology as possibly participating in this pathway (*3*). In addition, there is a minor, short-patch MMR system in *S. pombe* that recognizes all possible base combinations, including C·C mispairs (*25*, *26*). Recently, the *S. pombe* nucleotide excision repair genes *rhp14*$^+$, *swi10*$^+$, and *rad16*$^+$ have been identified as components of the short-patch MMR system and have been shown to function independently of *msh2* and *pms1* (*27*). *S. pombe* must therefore possess multiple pathways for mismatch repair in vivo.

This study characterizes the ability of Uve1p to recognize and incise duplex DNA-containing mismatches, IDLs, and hairpins. In addition, a determinant of the strand specificity for Uve1p-mediated DNA cleavage is examined. Evidence that the 3′ terminus of the strand containing a mismatch is involved in directing the strand specificity of Uve1p is presented, and the implications for such strand discrimination are discussed.

MATERIALS AND METHODS

Protein Purification. GΔ228-Uve1p was purified by glutathione−Sepharose affinity chromatography as previously described (*13*). *S. cerevisiae* cells expressing GΔ228-Uve1p were grown until mid-log phase and then induced with 0.5 mM $CuSO_4$ for 1 h. Cells were lysed by mechanical disruption using glass beads, and the cell lysate was centrifuged at 45000*g* for 20 min. The soluble fraction was loaded onto a glutathione−Sepharose column, and the column was washed with phosphate-buffered saline (pH 7.4). The bound protein was eluted with 10 mM glutathione in 50 mM Tris (pH 7.4) as previously described (*13*). Purified, untagged Δ228-Uve1p was prepared by thrombin cleavage of GΔ228-Uve1p bound to glutathione−Sepharose as previously described (*13*). The enzyme preparation was nearly homogeneous as determined by SDS−PAGE analysis. The purified enzyme fractions were stored in 10% glycerol at −80 °C. Purified *E. coli* endonuclease V (*28*, *29*) was a gift from Y. W. Kow (Atlanta, GA).

Substrate Preparation. The DNA sequences of the oligonucleotide substrates used in this study are presented in Figure 1 and Table 1. Oligonucleotides were synthesized either by Operon, Inc. (Alameda, CA), or by the Emory University Microchemical Facility (Atlanta, GA). Oligonucleotides were gel purified and their sequences confirmed by DNA sequence analysis prior to use. Oligonucleotides were 5′-end-labeled with T4 polynucleotide kinase using 50 μCi of [γ-^{32}P]ATP (Amersham, 3000 Ci/mmol) as previously described (*30*). The 3′-end-labeled oligonucleotides were prepared by incubating 10 pmol of the oligonucleotide with

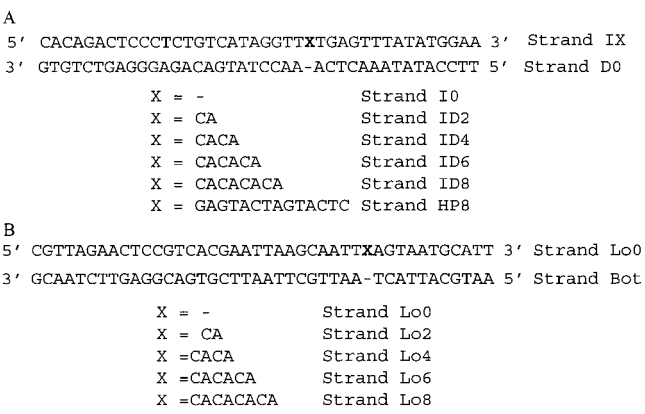

FIGURE 1: Potential IDL substrates for Uve1p. Sequences of the oligonucleotide strands utilized to generate the heteroduplex substrates used in this study. Names of individual oligonucleotide strands are indicated beside each duplex. Two different sequence types (I and Lo) were used. (A) Duplex type ID substrates containing IDL loops 0, 2, 4, 6, 8, and 16 nt in length. (B) Duplex type LD substrates containing IDL loops 0, 2, 4, 6, and 8 nt in length.

50 μCi of [α-^{32}P]dideoxyATP (Amersham, 3000 Ci/mmol) and 10 units of terminal deoxynucleotidyl transferase (Promega) as previously described (*19*). The end-labeled oligonucleotides were annealed to the appropriate complementary strand, and the duplex DNA substrate was purified by gel electrophoresis on a native (nondenaturing) polyacrylamide gel. The IDL or hairpin nature of the DNA substrates was also confirmed by monitoring DNA strand cleavage by purified *E. coli* endonuclease V, which cleaved the duplexes (data not shown) in a pattern specific for this enzyme (*28*, *29*).

Endonuclease Assays. Standard reaction mixtures contained approximately 200 fmol of end-labeled oligonucleotide substrate and 200 ng of GΔ228-Uve1p in buffer containing 20 mM Hepes (pH 6.5), 10 mM $MgCl_2$, 1 mM $MnCl_2$, and 150 mM NaCl (10−20 μL final volume). Reactions were carried out for 15 min at 37 °C. The reaction products were extracted with an equal volume of a phenol/chloroform/isoamyl alcohol mixture (25:24:1), followed by ethanol precipitation. The precipitated reaction products were resuspended and analyzed on a 20% denaturing (7 M urea) DNA sequencing type gel as previously described (*13*). Uncleaved DNA substrate and the cleaved reaction products were analyzed and quantified by phosphorimager analysis (Molecular Dynamics model 445SI) and/or autoradiography. Base-specific chemical cleavage DNA sequencing reaction products were included in the gels as size markers.

Antibody Inhibition. The N-terminal glutathione *S*-transferase tag was removed from GΔ228-Uve1p by thrombin cleavage, and Δ228-Uve1p was purified as described previously (*13*). Rabbit polyclonal antibodies were raised against purified Δ228-Uve1p. GΔ228-Uve1p was incubated with immune or preimmune sera for 45 min at 4 °C and then incubated with the indicated end-labeled duplex oligonucleotides for Uve1p assay as described above. The reaction products were analyzed as described above.

RESULTS

Uve1p Recognizes Loops but Not Hairpin Structures in Duplex DNA. Uve1p was initially described as a UV damage-

Table 1: Sequences of Oligonucleotides Used in Strand Specificity Experiments[a]

Oligo Name	Sequence	Strand Designation
1	5'CGTTACAAGTCCGTCACGAATTAAGCAATTCGTAA**C**GCATT3' 3'GCAATGTTCAGGCAGTGCTTAATTCGTTAAGCATT**A**CGTAA5'	Strand C(6) Strand A(36)
2	5'CGTTAC**A**AGTCCGTCACGAATTAAGCAATT**C**GTAACGCATT3' 3'GCAATGTTCAGGCAGTGCTTAATTCGTTAA**A**CATTGCGTAA5'	Strand C(11) Strand A(31)
3	5'CGTTACAAGTCCGTCACGAATTAAG**C**AATTCGTAACGCATT3' 3'GCAATGTTCAGGCAGTGCTTAATTC**A**TTAAGCATTGCGTAA5'	Strand C(16) Strand A(26)
4	5'CGTTACAAGTCCGTCACGA**C**TTAAGCAATTCGTAACGCATT3' 3'GCAATGTTCAGGCAGTGCT**A**AATTCGTTAAGCATTGCGTAA5'	Strand C(22) Strand A(20)
5	5'CGTTACAAGTCCGT**C**ACGAATTAAGCAATTCGTAACGCATT3' 3'GCAATGTTCAGGC**A**ATGCTTAATTCGTTAAGCATTGCGTAA5'	Strand C(27) Strand A(15)
6	5'CGTTACAAG**C**CCGTCACGAATTAAGCAATTCGTAACGCATT3' 3'GCAATGTTC**A**GGCAGTGCTTAATTCGTTAAGCATTGCGTAA5'	Strand C(32) Strand A(10)
7	5'CGTT**C**CAAGTCCGTCACGAATTAAGCAATTCGTAACGCATT3' 3'GCAA**A**GTTCAGGCAGTGCTTAATTCGTTAAGCATTGCGTAA5'	Strand C(37) Strand A(5)
Duplex AB	5'TGCACGGACCTCGAGAGAACCGAACGATCCGGATGTGATCATGGGACAGAATTCGCACACTGCACTGCACGATAGTCTC3' 3' TCTTGGCTTGCTAGGCCTACACTA**C**TACCCTGTCTTAAGCGTGTGACGT5'	Strand A Strand B
Duplex AC	5'TGCACGGACCTCGAGAGAACCGAACGATCCGGATGTGATCATGGGACAGAATTCGCACACTGCACTGCACGATAGTCTC3' 3' TCTTGGCTTGCTAGGCCTACACTA**C**TACCCTGTCTTAAGCGTGTGACGTGCTATCAGAG5'	Strand A Strand C
Duplex AD	5'TGCACGGACCTCGAGAGAACCGAACGATCCGGATGTGATCATGGGACAGAATTCGCACACTGCACTGCACGATAGTCTC3' 3'ACGTGCCTGGAGCTCTCTTGGCTTGCTAGGCCTACACTA**C**TACCCTGTCTTAAGCGTGTGACGT5'	Strand A Strand D
Duplex AE	5'TGCACGGACCTCGAGAGAACCGAACGATCCGGATGTGATCATGGGACAGAATTCGCACACTGCACTGCACGATAGTCTC3' 3'ACGTGCCTGGAGCTCTCTTGGCTTGCTAGGCCTACACTA**C**TACCCTGTCTTAAGCGTGTGACGTGACGTGCTATCAGAG5'	Strand A Strand E

[a] C·A and C·C mispairs are indicated in bold face.

dependent, ATP-independent DNA endonuclease (*14*). We previously described the overexpression, purification to apparent homogeneity, and characterization of a stable, fully active GST-tagged truncated version of the enzyme (GΔ228-Uve1p) that lacks its N-terminal 228 amino acids (*13*). The truncated version of the enzyme has been utilized for in vitro biochemical experiments because the full-length purified protein is very unstable (*13*, *19*, *20*). GΔ228-Uve1p recognizes and incises heteroduplex DNA 5′ to the site of a mismatch (*18*). *S. pombe uve1* null mutants have a spontaneous mutator phenotype implicating Uve1p in mismatch repair processes (*18*). Here we investigate the ability of Uve1p to recognize and process IDL structures in duplex DNA as well as the determinants of the strand specificity of Uve1p.

The ability of GΔ228-Uve1p to process DNA duplexes containing IDL and hairpin structures was investigated with a series of oligonucleotide substrates containing 2−8 nt loops or an 8 bp hairpin structure (Figure 1). The DNA sequence and structures of the substrates are shown in Figure 1. Strands I0, I2, I4, I6, I8, or HP8 were 3′-end-labeled and annealed to strand D0. This annealing reaction formed duplexes ID0, ID2, ID4, ID6, ID8, and HPD8, with 0, 2, 4, 6, and 8 nt loops and an 8 bp hairpin, respectively. The substrates were treated with GΔ228-Uve1p (Figure 2, even lanes) or with buffer alone (Figure 2, odd lanes). No DNA strand cleavage was observed for the normal homoduplex substrate ID0 (Figure 2A, lane 2). In contrast, GΔ228-Uve1p cleaves heteroduplexes containing insertions of 2 or 4 unpaired nucleotides (Figure 2A, lanes 4 and 6). However, the enzyme did not cleave duplex ID6 or ID8 containing larger loops of 6 or 8 nucleotides, respectively (Figure 2C, compare lanes 2, 4, and 6).

The results shown in Figure 2 indicate that GΔ228-Uve1p produces several cleavage products from the strand containing a 2 or 4 nt unpaired loop. The sites of cleavage correspond to endonucleolytic cleavage of duplexes ID2 and ID4 a distance of 1 (position a), 15 (position b), and 16 (position c) nucleotides 5′ to the IDL (Figure 2A, lanes 4 and 6). The major site of Uve1p-mediated cleavage on duplex ID2 was observed to be at positions b and c, whereas the major site of cleavage on duplex ID4 was observed to be at position a. The cleavages at positions a−c were also observed when the DNA substrate was labeled at the 5′ terminus of the strand containing the loop (data not shown), which confirms that Uve1p cleaves endonucleolytically at these sites. Uve1p did not cleave DNA containing a base insertion within the context of a small hairpin structure (Figure 2B, lane 2).

Previous studies indicate that Uve1p has a wide substrate specificity, including bipyrimidine UV photoproducts, base mispairs, abasic sites, and platinum GG diadducts in duplex DNA (*18*−*20*). For each of these lesions, GΔ228-Uve1p cleaves endonucleolytically immediately, 1 or 2 nt 5′ to the site of base damage. The cleavage events at positions b and c (Figure 2A) located 15 and 16 nt 5′ to the site of the loop of substrate ID2 or ID4 have been observed only with these types of DNA substrates containing small base insertions. Because cleavage events at similar distances 5′ to the site of DNA damage were not observed in earlier studies, it seemed possible that the observed distal cleavage events were the result of a sequence-specific structure formed by the particular duplex used in this experiment. Therefore, the generality of this cleavage pattern was tested using IDL substrates within a different DNA sequence context.

DNA substrates were constructed by annealing oligonucleotides Lo0, Lo2, and Lo4 to strand Bot to generate duplexes LD0, LD2, and LD4 with loops of 0, 2, and 4 nt, respectively (Figure 1B). The strands which form a loop in the duplex (LD0, LD2, and LD4) were labeled on either the 3′ (Figure 3A) or the 5′ (Figure 3B) end. These substrates were incubated with purified GΔ228-Uve1p or with buffer, and the reaction products were analyzed on a 20% denaturing polyacrylamide gel (Figure 3). GΔ228-Uve1p cleaved duplexes LD2 and LD4 with base insertions of 2 or 4 nt. However, as observed previously with substrates ID6 and ID8, DNA substrates with 6 (LD6) or 8 (LD8) nt insertions were not cleaved by Uve1p (Figure 2C and data not shown). These results indicate that GΔ228-Uve1p recognizes and cleaves duplex DNA with loops of 2 or 4 nt but not IDL structures of 6 or 8 nt insertions or certain hairpin structures. The extent of GΔ228-Uve1p-mediated cleavage observed with IDL substrates containing 2 and 4 nt insertions was

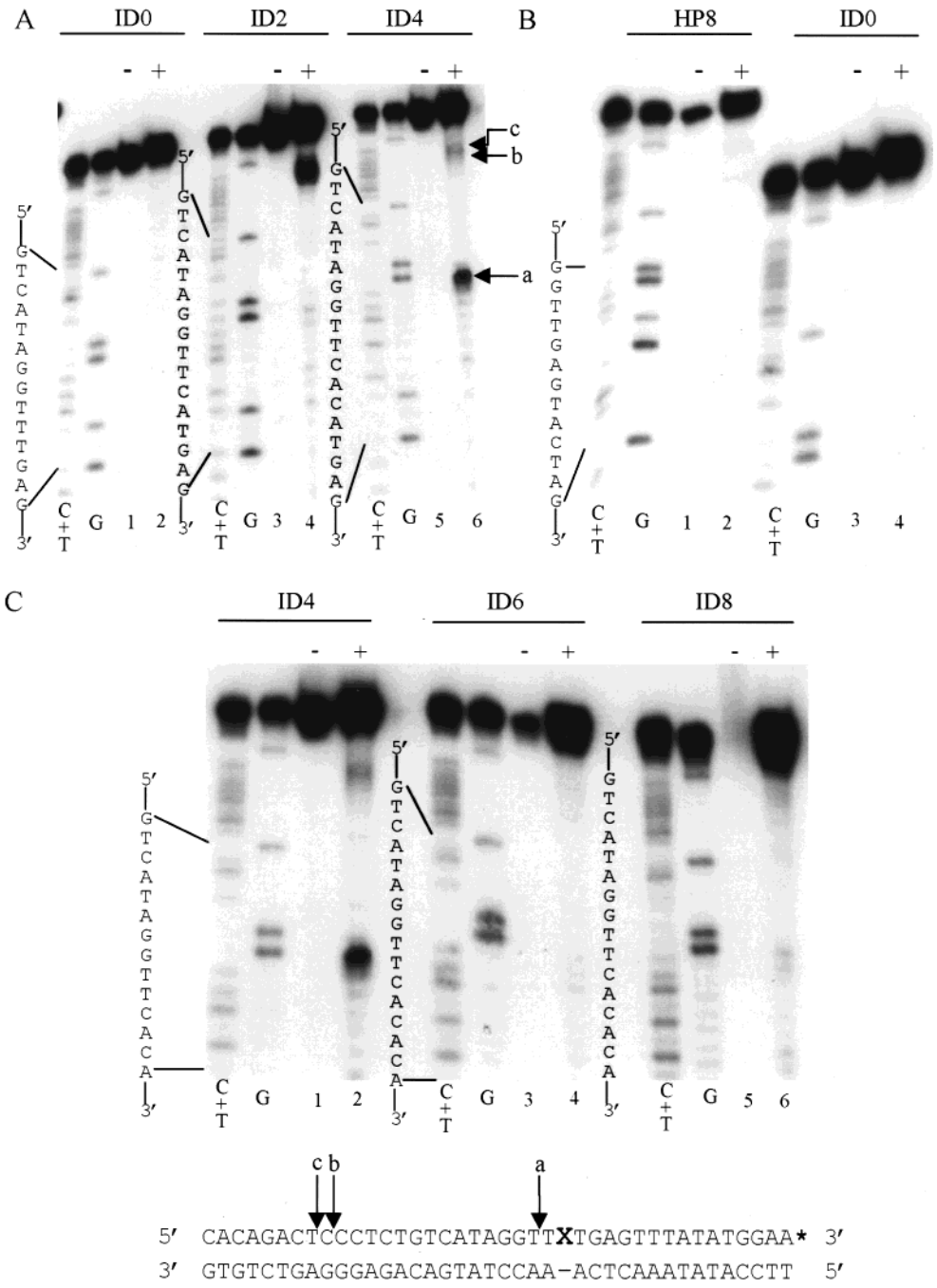

FIGURE 2: Uve1p recognizes small IDLs but not large IDLs or hairpin structures. Cleavage reactions were carried out with 3′-end-labeled DNA duplexes under standard reaction conditions, and products were analyzed on DNA sequencing gels as described in the text. Arrows (a−c) indicate GΔ228-Uve1-mediated DNA cleavage products. G and C+T base-specific chemical cleavage DNA sequencing ladders were run in adjacent lanes as nucleotide position markers. The 3′-end-labeled (*) core substrate sequence is shown at the bottom of the figure with Uve1p cleavage sites a−c indicated (X corresponds to IDL sequences shown in Figure 1A). (A) The 3′-end-labeled duplexes ID0, ID2, and ID4, (B) ID0 and HP8, and (C) ID4, ID6, and ID8 were treated with GΔ228-Uve1p [even-numbered (+) lanes] or buffer [odd-numbered (−) lanes].

approximately the same as that previously observed for a duplex DNA substrate containing a C·A mispair (*18*). Furthermore, for each of these IDL-containing duplexes, no cleavage of the opposite strand was observed.

Antibody Inhibition of Uve1p Incision of an IDL-Containing Substrate. To further investigate the involvement of Uve1p in the recognition and cleavage of substrates containing short IDL structures, antibody inhibition experiments were conducted. Rabbit polyclonal antibodies against Δ228-Uve1p (Materials and Methods) were generated. GΔ228-Uve1p was preincubated with 40−160 μg of preimmune or immune serum, and endonuclease assays with 3′-end-labeled substrate ID2 were carried out under standard reaction conditions. Preincubation of GΔ228-Uve1p with this antibody significantly decreases the extent of GΔ228-Uve1p-mediated cleavage of substrate ID2 in a concentration-dependent manner (Figure 4, lanes 2−5). In contrast, preincubation with preimmune serum did not inhibit the GΔ228-Uve1p-mediated endonuclease at any concentration that was tested (Figure 4, lanes 6−9). Western blot analysis of the purified GΔ228-Uve1p with the immune serum produced a single band, indicating that the antibody does

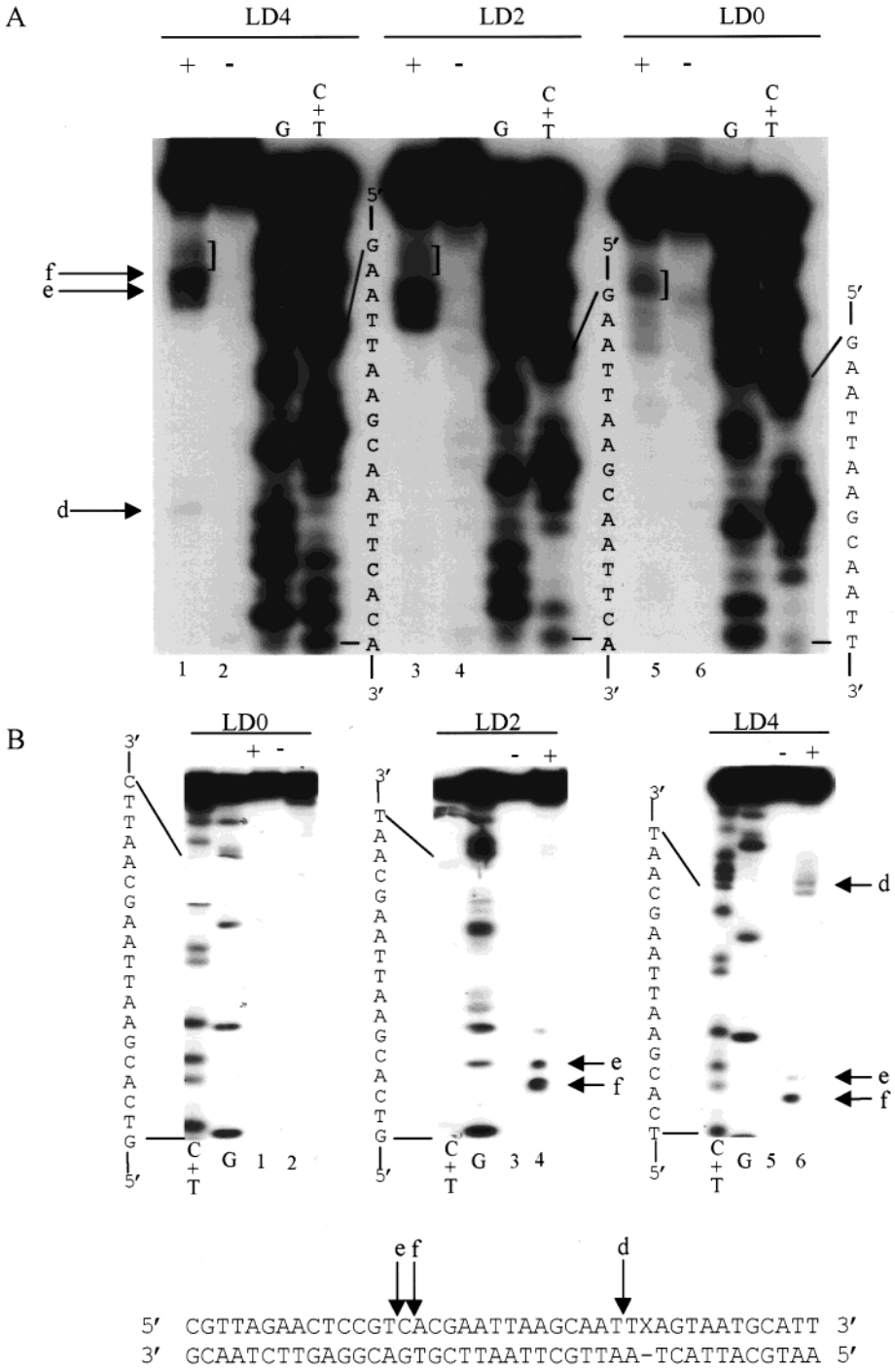

FIGURE 3: Uve1p recognition of short IDL structures within a different sequence context. Cleavage reactions were carried out with (A) 3′-end-labeled or (B) 5′-end-labeled DNA duplexes containing IDLs 0 (LD0), 2 (LD2), and 4 (LD4) nt in length and GΔ228-Uve1p as described in the text. DNA cleavage products were analyzed on sequencing type gels. Arrows d−f indicate DNA strand scission products produced by cleavage events occurring 1, 15, and 16 nt 5′ from the IDL site, respectively. G and C+T base-specific chemical cleavage DNA sequencing ladders were run in adjacent lanes as nucleotide position markers. The core sequence of the substrate is shown at the bottom of the figure where X corresponds to the IDL sequence depicted in Figure 1. In panel A, the vertical bracket above arrows e and f indicates nonspecific cleavage of the substrates and is not observed with 5′-end-labeled substrates.

not cross-react with any trace contaminating proteins in the purified GΔ228-Uve1p preparation used in these experiments (data not shown). The specific inhibition of the IDL cleavage activity of GΔ228-Uve1p by the antibody suggests that the observed cleavage of the IDL-containing substrates was associated with GΔ228-Uve1p.

Strand Specificity of Uve1p. Previous studies have demonstrated that Uve1p recognizes and processes UV photoproducts and all 12 bp mispair combinations in a similar manner (*13*, *18*, *19*). This study demonstrates that Uve1p is also capable of recognizing small IDL structures. We attempted to determine what features of known Uve1p substrates conferred specificity with respect to DNA strand cleavage preference. The substrate specificity of Uve1p in vitro suggests that it could potentially participate in repair of mismatches or IDL structures in vivo. If this is the case, then it would be important to determine how the enzyme might distinguish between the "correct" and "incorrect" base

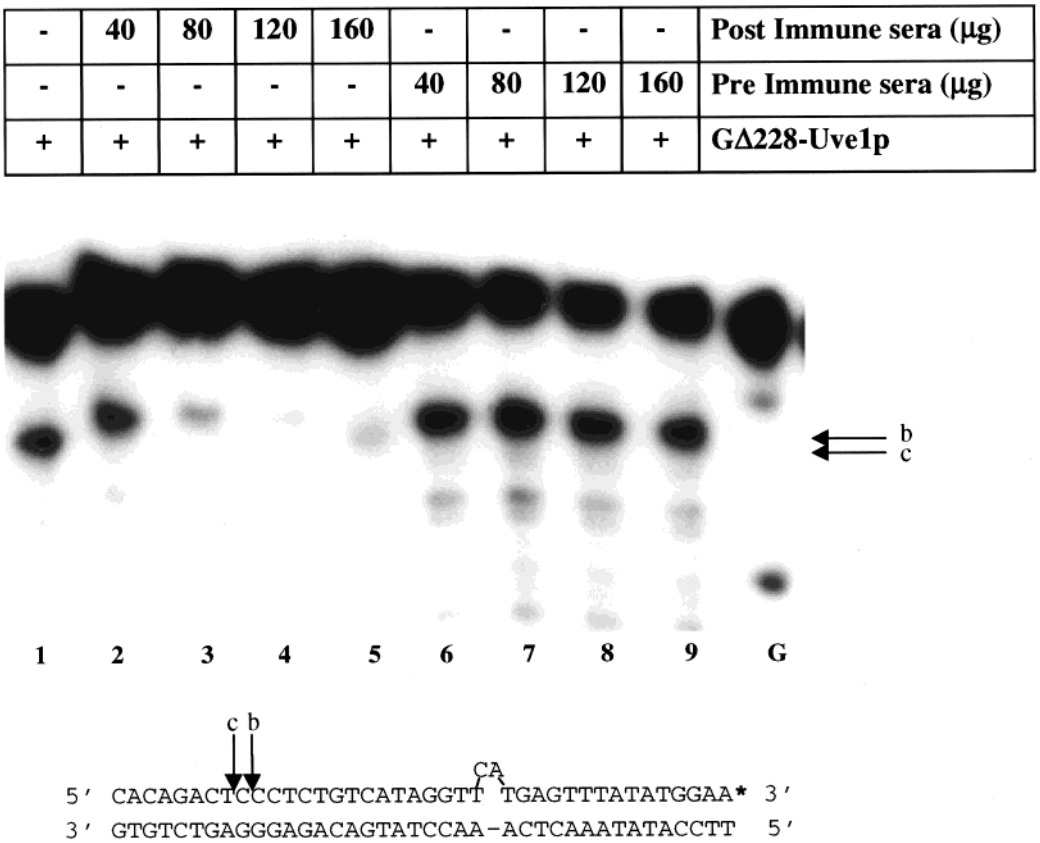

FIGURE 4: Antibody inhibition of Uve1p-mediated activity in an IDL-containing substrate. GΔ228-Uve1p was preincubated with increasing amounts (40−160 μg) of immune serum (lanes 2−5) or preimmune serum (lanes 6−9) prior to incubation with 3′-end-labeled substrate ID2 (sequence indicated at the bottom). The reaction products were analyzed on a 20% denaturing polyacrylamide gel as described in Materials and Methods. Arrows b and c correspond to GΔ228-Uve1p-mediated DNA cleavage events 15 and 16 nt 5′ to the IDL site, respectively. In this gel, the two bands representing distinct cleavage events at positions b and c are poorly resolved from each other.

in a mispair. One possibility is that the proximity of one of the mispaired bases to either the 3′ or the 5′ terminus might direct strand specificity for cleavage. For example, *E. coli* endonuclease V is a mismatch endonuclease that displays strong strand specificity, preferring to cleave the strand containing the mispaired base closest to the 5′ terminus (*28*). We tested the possibility that the strand specificity of GΔ228-Uve1p is affected by the nature of the terminus closest to the mispair.

A series of oligonucleotides (41-mers) were synthesized which form DNA duplexes with a single C·A mispair at different positions within the sequence and at different distances from the termini of the linear duplex DNA molecule (Table 1). The C-containing strand was 3′-end-labeled, and the relative cleavage activity of GΔ228-Uve1p on this strand was quantified (Figure 5A). DNA cleavage was relatively inefficient at the C on this strand when located 37 nt from the 3′ terminus; the extent of cleavage gradually increased to a maximum when located 16 nt from the 3′ terminus, and cleavage was undetectable when located 6 nt from the 3′ terminus. A similar result was obtained when the A-containing strand of the C·A mispair was analyzed (Figure 5B). These results suggest that the cleavage efficiency of Uve1p at a C·A mismatch may be sensitive to the proximity of the mispaired base to the 3′ terminus. However, in this experiment the flanking DNA sequence context of the C·A mispair varies at different positions within the DNA duplex, which may also influence the cleavage efficiency of Uve1p.

To gain further insight into the strand preferences of Uve1p-mediated DNA cleavage, four duplex DNA substrates (AB, AC, AD, and AE) were designed to test the cleavage efficiency of Uve1p at a C·C mismatch within the same sequence context but at different distances from 3′ and 5′ termini (Table 1). The length and sequence of the "A" strand of these duplexes were identical in all cases, and the length of the complementary strand (B, C, D, or E) was varied. The cleavage efficiency of GΔ228-Uve1p on strands B−E in substrates AB, AC, AD, and AE was quantified (Figure 5C). Substrates AB and AC in which the C·C mispair was 25 nt from the 3′ terminus were cleaved by GΔ228-Uve1p with a similar efficiency. In contrast, the efficiency of cleavage of substrates AD and AE in which the C·C mispair was 40 nt from the 3′ terminus was about 2-fold lower. Moreover, the efficiency of cleavage of the duplex substrate appeared to be independent of the distance of the mismatch from the 5′ terminus (compare AB with AC and AD with AE). These results are consistent with the notion that the efficiency of cleavage of a mispaired base by GΔ228-Uve1p is sensitive to its proximity to the 3′ terminus of that strand.

DISCUSSION

Uve1p is an ATP-independent endonuclease that incises DNA at sites of cyclobutane pyrimidine dimers and 6−4 photoproducts (*14*). In addition to UV photoproducts, the enzyme recognizes platinum−DNA GG diadducts, abasic sites, and base−base mispairs in duplex DNA (*18*−*20*). The chemical nature of the lesion, as well as a combination of

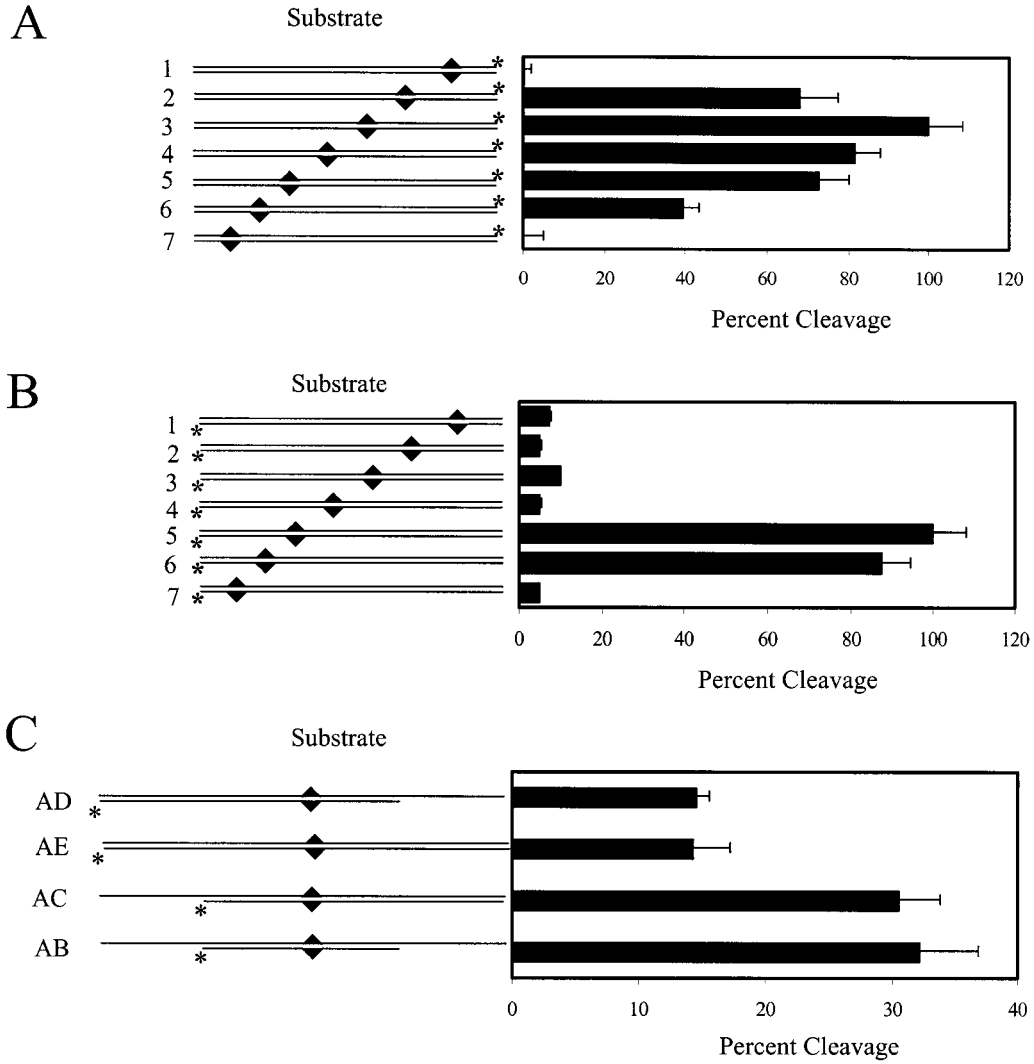

FIGURE 5: Strand specificity of Uve1p which is influenced by the proximity of the mispaired base to the 3′ terminus. DNA substrates used in this experiment are shown in Table 1. Number and letter designations adjacent to substrates correspond to the oligonucleotide names given in Table 1. Duplex substrates were 3′-end-labeled (*) on (A) C-containing or (B) A-containing strands and incubated with GΔ228-Uve1p as described in the text. The extent of DNA strand scission (expressed as the percent of total substrate cleaved) was quantified by phosphorimager analysis. (C) Substrates AB, AC, AE, and AD were 3′-end-labeled and incubated with GΔ228-Uve1p under standard conditions (Materials and Methods). The percent cleavage was quantified by phosphorimager analysis. The error bars indicate the mean ± the standard deviation obtained from three separate experiments.

various structural factors, may determine the substrate preference of Uve1p (*19*).

Repetitive sequences in DNA are capable of forming IDL structures during replication, which can lead to insertion or deletion mutations (*4*, *5*). MMR systems in both prokaryotes and eukaryotes are utilized for the repair of such IDL structures (*6*, *8*, *11*). This work demonstrates that Uve1p incises small IDL structures in DNA, indicating that it may function in maintaining the stability of repetitive sequences in the genome. Our results also show that Uve1p cleaves DNA 15−16 nucleotides 5′ to the IDL structure (Figures 2 and 3). The reasons for such a distal cleavage event are unknown; however, one possibility is that Uve1p causes a distortion in the DNA when it binds to a mismatch and that this distortion leads to the distal cleavage event. In this case, cleavage at the distal site would result from a conformation of Uve1p and DNA unique to the complex formed by Uve1p at an IDL.

The substrate specificity of Uve1p, including IDL structures as well as base mispairs, is not a unique property. *E. coli* endonuclease V, spinach nuclease SP, and T4 endonuclease VII also share these properties in that they recognize and cleave DNA-containing base mispairs and small IDLs (*31*−*33*). However, these enzymes also differ from each other with respect to the sites and extent of cleavage in proximity to the DNA distortion as well as their recognition of other types of DNA damage and other types of DNA structures. In addition, the physical characteristics and environment in which optimal activity is observed vary considerably.

The study presented here also demonstrates that Uve1p does not recognize hairpin type structures. In this respect, Uve1p is distinct from *E. coli* endonuclease V, but similar to *S. cerevisiae* insertion mismatch recognition activity (*34*). IDL structures that form hairpins are considered to be poor substrates for repair (*35*) and demonstrate a low rate of repair in vivo in yeast (*36*). It is also important to note that the recombinant GST-tagged, truncated version of Uve1p has been utilized in all these experiments which may or may not reflect the substrate specificity of the native enzyme in vivo.

To contribute to mismatch repair in vivo in a manner that maintains genomic stability, Uve1p requires a mechanism for strand discrimination. Our results suggest that during mismatch recognition and cleavage, the strand preference of Uve1p is determined by the proximity of a mispaired base to the 3′ terminus. This strand selection mechanism may reflect the fact that during DNA replication newly generated base misincorporations in the lagging strand would be close to the DNA 3′ terminus associated with the replication fork. In this way, repair of mispair and IDL structures by Uve1p could contribute to genomic stability in vivo.

Recent studies indicate that DNA repair pathways for damaged DNA bases are also in some cases involved in mismatch repair (*3*, *37*). Rad1p, a nucleotide excision repair protein of *S. cerevisiae*, is thought to be important for the repair of DNA loops (*38*). Biochemical as well as genetic evidence indicates that the Rad1p/Rad10p endonuclease is absolutely required for nucleotide excision repair of UV-damaged DNA and that it also functions in genetic recombination (*39*−*41*). *S. pombe* Rad16p and Swi10p are the homologues of the yeast Rad1p and Rad10p. Mutations in these two proteins reduce the frequency of mating type switching and also confer a radiation-sensitive phenotype (*42*−*44*). There is also evidence indicating that mismatch repair proteins recognize various types of DNA adducts (*45*). Thus, it is conceivable that Uve1p is an endonuclease that may participate in the repair of UV-induced DNA damage as well as DNA replication-associated mismatch or IDL lesions.

The *S. cerevisiae* MutS homologue Msh2p exists in a complex with NER proteins, indicating a biochemical and genetic basis for these proteins to function in common processes (*46*). Mutations in *MSH2* increase the UV sensitivity of NER-deficient *S. cerevisiae* strains, and such mutations are epistatic to the mutator phenotype observed in NER-deficient strains (*46*). Thus, there is an accumulating body of evidence indicating that pathways designed for the repair of damaged DNA and mismatch repair interact, but the biological significance and/or importance of such "cross-talk" is not yet fully understood. We propose that Uve1p is an example of a repair enzyme with a wide substrate specificity that may be involved in interactions between DNA excision repair and mismatch repair pathways.

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