DNA substrate specificity of DNA helicase E from calf thymus

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ABSTRACT

DNA helicase E from calf thymus has been characterized with respect to DNA substrate specificity. The helicase was capable of displacing DNA fragments up to 140 nucleotides in length, but was unable to displace a DNA fragment 322 nucleotides in length. DNA competition experiments revealed that helicase E was moderately processive for translocation on single strand M13mp18 DNA, and that the helicase would dissociate and rebind during a 15 minute reaction. Comparison of the rate of ATPase activity catalyzed by helicase E on single strand DNA substrates of different lengths, suggested a processivity consistent with the competition experiments. The helicase displayed a preference for displacing primers whose 5' terminus was fully annealed as opposed to primers with a 12 nucleotide 5' unannealed tail. The presence of a 12 nucleotide 3' tail had no effect on the rate of displacement. DNA helicase E was capable of displacing a primer downstream of either a four nucleotide gap, a one nucleotide gap or a nick in the DNA substrate. Helicase E was inactive on a fully duplex DNA 30 base pairs in length. Calf thymus RP-A stimulated the DNA displacement activity of helicase E. These properties are consistent with a role for DNA helicase E in chromosomal DNA repair.

INTRODUCTION

DNA helicases perform strand separation reactions in a variety of processes including DNA replication, repair, and recombination (1,2). Helicases generally bind to single stranded (ss)¹ DNA and translocate along the DNA into a duplex region disrupting the hydrogen bonds between the two DNA strands (3).

We recently have purified and partially characterized a DNA helicase (hel E) from calf thymus (4). Hel E is a 104 Kda monomer capable of displacing a 24 nucleotide primer from ss M13mp18 DNA. It translocates in the 3' to 5' direction with respect to the template strand, and preferentially utilizes ATP or dATP as a source of energy. These physical and enzymological characteristics distinguish hel E from the four helicases isolated from calf thymus by Thömmes *et al.* (5), only one of which

translocated in the 3' to 5' direction. Two human helicases identified by Tuteja *et al.* (6,7) and the human helicase isolated by Seo *et al.* (8) also have distinct properties which distinguish these helicases from hel E.

DNA hel E was initially identified as an activity present in preparations of DNA polymerase ϵ (pol ϵ). The purification procedure employed was designed to minimize disruption of the protein-protein interactions (9). Our initial report on hel E demonstrated partial co-elution of pol ϵ with hel E over the chromatographic matrices used during purification (4). These data suggest that hel E may form a loose physical complex with DNA pol ϵ . Recent work from this laboratory has demonstrated a functional coordination between hel E and pol ϵ resulting in DNA synthesis through a downstream primer dependent on helicase activity (10). The functional interaction with hel E was demonstrated to be specific for pol ϵ . The results suggested that hel E may participate with pol ϵ at the eukaryotic replication fork or in DNA repair. In this report we have characterized the DNA substrate specificity of hel E in order to assign a possible role for hel E in DNA repair or replication. The DNA substrates employed in this study were designed to simulate DNA structures that may be encountered in DNA replication and repair. The implications of the ability of hel E to function on the DNA substrates are discussed.

MATERIALS AND METHODS

Materials

Unlabeled nucleotides were from Pharmacia (Piscataway, NJ) and radio-labeled nucleotides were from New England Nuclear (Boston, MA). Synthetic DNA oligonucleotides were purchased, gel purified, from Genosys (The Woodlands, TX) and their sequences are shown in Table I. DNA modifying enzymes and Sequenase (ver. 2.0) were from United States Biochemical Corp. (Cleveland, OH). All other reagents were purchased from standard suppliers.

Enzymes

DNA hel E was purified from calf thymus essentially as previously described to a displacement specific activity of approximately 1500 units/mg and ATPase specific activity of

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2500 units/mg (4). One unit of helicase activity will displace 50% of a 24 nucleotide DNA primer from 100 fmol of ssM13mp18 DNA in a 30 min. reaction at 37°C. Calf thymus RP-A was purified by a modification of the procedure described by Thömmes *et al.* (5). It was confirmed to be authentic RP-A by Western blot analysis using a polyclonal antibody generated against human RP-A, kindly provided by Dr. Marc S. Wold (University of Iowa).

Enzyme assays

DNA helicase assays were performed in 20 mM Hepes pH 7.0, 50 mM NaCl, 2 mM MgCl₂, 1 mM DTT, 5 mM ATP in a final volume of 20 μ l. Reactions contained 15 fmol of DNA substrate and were incubated for 15 min. at 37°C unless stated otherwise. Products were analyzed by 10% native polyacrylamide gel electrophoresis, and visualized by autoradiography as previously described (10).

Assays for DNA dependent ATPase were performed in a final volume of 20 μ l containing 20 mM HEPES (pH 7.0), 1 mM DTT, 4.0 mM MgCl₂ and [γ -³²P]ATP (0.4 mM, 500 cpm/pmol). Reactions were stopped by the addition of 0.5 ml ice cold 4% activated charcoal. Samples were sedimented for 20 min. at 12,000 $\times$ g at 4°C. The supernatant was removed and Cherenkov counted to determine the release of [³²Pi].

DNA substrates

The DNA substrates employed in this study are shown in Table 1. DNA oligonucleotides were 5' phosphorylated using [γ -³²P]ATP (100 μ Ci, 3000 Ci/mmol) and T4 polynucleotide kinase. Labeled primers were annealed to ssM13mp18 in a 2:1 ratio, and duplex DNA's were purified by 2 ml Sepharose CL-2B chromatography.

The 4 nucleotide gap and 1 nucleotide gap DNA substrates were constructed as follows: The (–20) 17-mer primer was annealed to the 54-mer synthetic snapback DNA at a 2:1 molar ratio at 65°C for 10 min. then cooled to room temperature over 3 hours. Labeling of the downstream primer was accomplished by the addition of one nucleotide using [α -³²P]dGTP (100 μ Ci, 3000 Ci/mmol) and 5 units of Sequenase (version 2.0) at 37°C for 15 min. The one nucleotide gapped DNA substrate was constructed by adding dATP (50 μ M) to the extension reaction. This served to extend the upstream 3'-OH by 3 nucleotides. The reactions were stopped by the addition of EDTA to a final concentration of 10 mM. The resulting product was purified by sedimenting through Sephadex G-50 spin columns.

The nicked DNA substrate was constructed by annealing the (–20) 17-mer primer and (–47) 24-mer primer to the synthetic 44-mer in a 2:1 ratio of each primer to template. Extension in the presence of dGTP and [α -³²P]dCTP resulted in radiolabeling of each primer and generation of a nick between the two primers. The fully duplex substrate was constructed by 5' [³²P] end-labeling the 30-mer with T4 polynucleotide kinase and annealing to an equal concentration of a complementary 30-mer.

M13mp18 was digested with *Hinf I* and *Pvu II* according to the manufacturer's specifications. The 137 bp *Hinf I* and 322 bp *Pvu II* fragments of M13mp18 positions 6904–7040 and 6053–6374, respectively, were excised from low melting point agarose, annealed to ssM13mp18, labeled by extension of three deoxynucleotides using Sequenase (version 2.0), [α -³²P]dGTP, dCTP, and dATP, for the *Hinf I* fragment and [α -³²P]dGTP for the *Pvu II* fragment. The DNA substrates were purified as described above.

Table 1. DNA Oligonucleotides

DNA	Sequence (listed 5' to 3')
(–20) 17-mer	GTAAAAACGACGGCCAGT
(–20) 18-mer	GTAAAAACGACGGCCAGTG
(–47) 24-mer	CGCCAGGGTTTCCAGTCACGAC
30-mer	GGGCGAATTCGAGCTCGGTACCCGGGGATC
44-mer	GCACTGGCCGTCGTTTTACGGTCGTGACTGGGAAA- ACCCTGGCG
5' tailed primer	TCAGTGCTGCAAGTAAACGACGGCCAGTG
3' tailed primer	GTAAAAACGACGGCCAGTGGGTTCTGAACGTA
5' + 3' tailed (42-mer)	TCAGTGCTGCAAGTAAACGACGGCCAGTGGGTTCT- GAACGTA
54-mer snapback	CACTGGCCGTCGTTTTACATTTGAGACGTCCCAGC- TTTTTTGCTGGGACGTCTC
74-mer	ACTGACTGACTGACTGACTGACTGACTGACTGAC- TGACTGACTGACCTCCGAGCTTGGCGTAATCATG- GTCAT

The DNA substrate containing various size duplex regions was constructed by annealing the (–20) 17mer to ss M13mp18 followed by extension with *E. coli* pol I Klenow fragment (13 units) and dCTP, dATP, dTTP, [α -³²P]dGTP for 30 min. at 30°C. The resulting product was purified by Sephadex G-50 spin column chromatography.

Single stranded M13mp18 was linearized following hybridization of a synthetic 20-mer complementary to the multicloning site, followed by digestion with *Bam HI* according to the manufacturer's specifications.

RESULTS

Length limitation of displacement by hel E

We have characterized the specificity of DNA hel E on a variety of DNA substrates. Hel E was purified based on the ability to displace a 24-mer annealed to M13mp18. We previously demonstrated displacement of a DNA fragment 58 nucleotide in length, from ssM13mp18 (4). We have taken two approaches to determine whether hel E is capable of displacing larger DNA fragments. The first involved gel purification of various size restriction fragments from double stranded M13mp18 and annealing these to ssM13mp18. A 140 bp *Hinf I* fragment was isolated, annealed, labeled and purified as described in 'Materials and Methods'. Fig. 1 panel A shows that hel E is capable of displacing the 140 nucleotide fragment. However, displacement required extensive incubation time and high levels of hel E compared to displacement of shorter DNA fragments. A 322 bp *Pvu II* fragment was also purified, and hel E was incapable of displacing this length DNA fragment (panel B). Both of the DNA fragments were analyzed for the potential to form secondary structures and no significant structures were found. A second approach to determine the length of duplex DNA hel E was capable of displacing involved constructing a DNA substrate containing various size duplex regions. This substrate was generated by extending a synthetic 17-mer hybridized to ssM13mp18 with *E. coli* pol I Klenow fragment and dCTP, dATP, dTTP, [α -³²P]dGTP. Using this substrate in displacement reactions revealed that hel E could displace DNA fragments several hundred nucleotides in length (panel C). The internally labeled DNA substrate was used at a concentration 50 fold lower than that used for the restriction fragments.

Figure 1. Length of Displacement by DNA hel E. The 137bp *Hinf I* (panel A) and 322 bp *Pvu II* (panel B) fragments of M13mp18 were purified, annealed to ss M13mp18 and 3' [32 P] labeled as described in 'Materials and Methods' and 15 fmol were used per reaction. The internally labeled DNA substrate (panel C) was constructed as described in 'Methods and Materials' and 0.3 fmol were used per reaction. Panel A; Lane 5 is a heat denatured control, and lane 6 is a no enzyme control. Panel B; Lane 1 is a no enzyme control and lane 2 is a heat denatured control. Panel C; Lane 1 is a heat denatured control, 20% of a full reaction; lane 2 is a no enzyme control. For all panels, the units of hel E are given above the Figure. Helicase reactions were performed for 60 min. at 37°C, and products analyzed by 8% native polyacrylamide gel electrophoresis.

Displacement of larger DNA fragments is only observed at a high hel E/DNA substrate ratio. Therefore, it appears that displacement of large DNA fragments requires multiple helicase molecules per DNA substrate. We note that the presence of hel E generates some products that appear to be larger than the heat denatured products. We considered that these might result from low level nuclease contamination in the hel E preparation. No nuclease is evident in the oligonucleotide displacement experiments, but these were performed at a much higher DNA concentration. Alternatively, the long products could result from the generation of multimers of displaced DNA segments, with or without bound hel E.

Processive motion on single stranded DNA

The processivity of helicase E was assessed in DNA substrate competition experiments. Hel E was incubated with increasing concentrations of either circular or linear ssM13mp18 DNA substrate in the absence of ATP. Preliminary nitrocellulose filter binding experiments demonstrated that hel E binds very efficiently to ss DNA in the absence of ATP (data not shown). Reactions were then initiated with ATP and ssM13mp18[32 P]18-mer. In the absence of competitor DNA, hel E displaced 80% of the input DNA primer. Incubation with increasing competitor DNA before the initiation of the reaction resulted in a decrease in displacement activity (Fig. 2A). At an equal concentration of competitor DNA to labeled DNA, 5 fmol, we observed 50% of the maximum displacement. At the highest level of competitor DNA, displacement was reduced to about 20%. This suggests that hel E can dissociate and rebind the DNA substrates throughout the reaction.

The converse experiment was also performed in which hel E was pre-bound to the DNA substrate containing the labeled primer. These reactions were initiated with ATP and unlabeled competitor DNA substrate. The results shown in Fig. 2B demonstrate a decrease in displacement with addition of unlabeled competitor. At an equal concentration of competitor DNA, displacement was decreased to about 60% of maximum. Additionally, at the highest level of competitor DNA the decrease was to 45–50% of maximum. Clearly, compared to the results in Fig. 2A, there was less inhibition of displacement. The

Figure 2. Competition for DNA hel E by ss M13mp18. Panel A: DNA hel E, 0.3 units, was incubated with increasing concentration of either closed circular (open symbols) or linear (filled symbols) ss M13mp18 DNA in the absence of ATP. Reactions were initiated with ATP and 5 fmol of M13mp18-[32 P]18-mer DNA substrate. Reactions were incubated for 15 min. at 37°C. Panel B: DNA hel E, 0.3 units, was incubated with 5 fmol of M13mp18-[32 P]18-mer DNA substrate. Reactions were initiated with ATP and increasing concentration of either closed circular (open symbols) or linear (filled symbols) ss M13mp18 DNA. Reactions were incubated for 15 min. at 37°C. The products were analyzed by 10% native polyacrylamide gel electrophoresis and visualized by autoradiography. Quantitation was performed by scanning laser densitometry, and results are presented as percent of maximum displacement.

difference between the two experiments is that in the former, hel E must dissociate from the unlabeled DNA substrate and move to the labeled DNA substrate before it can dissociate labeled primers. The lesser amount of inhibition in the latter experiment,

Figure 3. DNA dependent ATPase activity of hel E as a function of DNA substrate length. ATPase assays were performed as described in 'Materials and Methods'. A DNA concentration of 50 ng/reaction was used in these assays and was based on total nucleotide (not polymer molecules) to ensure that the total DNA available in the reaction was consistent. Reactions contained 0.3 units of hel E and the M13mp18, or the synthetic 24-mer or 74-mer. Reactions were incubated for 15 min. at 37°C, and results are expressed as pmol of ATP hydrolyzed.

therefore, must represent the displacement activity of pre-bound helicase molecules prior to their initial dissociation from the DNA with labeled primer. This suggests that approximately 20% of the pre-bound hel E molecules translocate along the M13 DNA, and displace a primer prior to helicase dissociation. This fraction of hel E molecules must have translocated processively from their original binding position on the single stranded DNA to the position of the primer. Evidently, the helicase is capable of processive motion for lengths that exceed the length of primers that it can displace (140 nucleotides), but that fall short of the length of M13. That the length of processive motion does not exceed the size of M13 is also suggested by the observation (Fig. 2) that circular and linear M13 are equally efficient competitors for hel E in displacement reactions. Overall, these results indicate that the helicase is moderately processive for translocation on single stranded DNA.

We have also estimated the processivity of hel E by analysis of the DNA dependent ATPase activity (Fig. 3). The ATPase activity was determined on both closed circular ssM13mp18 and linear ssM13mp18. Hel E displayed equal ATPase activity on each of the DNA substrates. The ATPase activity of hel E was also determined using synthetic 42 nucleotide and 74 nucleotide DNA substrates. Although the oligomers were present at the same nucleotide concentration as the M13 DNA, a decrease in ATPase activity was observed. Results with the oligomers suggest that when hel E encounters the end of a DNA substrate there is a decrease in ATPase activity. The decrease presumably results from stalling of motion at the end of the DNA substrate, or lack of ATPase during dissociation and the search for a new DNA substrate. If all rates are compared to that on the circular M13 DNA substrate, the inhibition is observed with the oligomers, but not with the much longer linearized M13 DNA. This suggests that a substantial proportion of the hel E molecules encounter the end of the oligomers but not of the long linear M13 DNA substrate. The processivity suggested by these results is substantially shorter than that of the M13, and in the same range or longer than that of the oligomers. These results are consistent with hel E displaying moderate processivity of motion on ss DNA substrates.

Interestingly, hel E displayed DNA dependent ATPase activity on double strand M13 DNA at approximately half the level of

Figure 4. Displacement of DNA fragments containing 5' and 3' mismatched tails. Panel A; The helicase substrates containing the 5' and/or 3' mismatched tails were incubated with 0.15 unit of hel E for 15 min. at 37°C. Panel B; Time course of displacement. The DNA substrates were incubated with 0.10 units of hel E for increasing time at 37°C. Panel C; Helicase titration of displacement of tailed DNA substrates. The helicase substrates containing the fully annealed 18 mer and 5' mismatched tail were incubated in the same reaction with increasing concentrations of hel E for 10 min. at 37°C. The products were analyzed by 10% (panel A and B) or 12% (panel C) native polyacrylamide gel electrophoresis followed by autoradiography. Quantitation was performed by scanning laser densitometry, and results are presented as percent of displaced fragment.

that observed with single strand DNA (data not shown). Nitrocellulose filter binding experiments revealed that hel E bound to the ds DNA at approximately half the level observed for ss DNA (data not shown).

Hel E is inhibited by replication fork structures

It has recently been demonstrated that DNA pol II, the yeast analog of pol ϵ , is required for DNA replication in yeast (11). Previous work from this laboratory demonstrated a functional interaction between hel E and DNA pol ϵ (10). Therefore, we determined whether the DNA displacement activity of hel E was enhanced by replication fork structures. The tailed primer DNA substrates were constructed as described in 'Materials and Methods'. Fig. 4A demonstrates that hel E was capable of displacing each primer to varying degrees. Hel E was inhibited by a DNA substrate containing a 5' unannealed tail, but the presence of a 3' tail had no effect on the level of displacement. The level of displacement of the primer containing both 5' and 3' tails was equal to that of the primer containing the 5' tail alone.

Figure 5. Hel E can displace primers from a 4 nucleotide, 1 nucleotide gap, and a nick in the DNA substrate, but not from a blunt end. Panel A; Reactions contained 15 fmol of the fully duplex DNA substrate. Lane 1 is a heat denatured control; lane 2, is a no enzyme control; lanes 3–6 contained 0.3, 0.6, 1.2, and 2.4 units of hel E respectively. Panel B; Reactions contained 15 fmol of the 4 nucleotide gap substrate (lanes 1–3), or 15 fmol of the 1 nucleotide gap substrate (lanes 4–6). Lanes 1 and 4 are the heat denatured controls; lanes 2 and 5 are the no enzyme controls; lanes 3 and 6 contained 0.15 units of hel E. Reactions were incubated for 15 min. at 37°C. Panel C; The reaction contained 15 fmol of the nicked DNA substrate and reactions were incubated for 15 min. at 37°C. Lane 1 is a heat denatured control; lane 2 is a no enzyme control; lane 3 contained 0.3 units of hel E. Reactions were incubated as above, and products analyzed as described in 'Materials and Methods'.

Kinetic analysis revealed that the rate of displacement for the fully annealed primer was faster than that for the 5' tailed primer, although both primers had the same maximum level of displacement following a 30 min. incubation (Fig. 4B). The fully annealed primer is 18 nucleotides long while the 5' tailed primer is 30 nucleotides long. Consequently these oligonucleotides are readily separated by native gel electrophoresis. This enabled us to perform an enzyme titration experiment, in a reaction containing both DNA substrates (Fig. 4C). The results demonstrate that hel E preferentially displaces the fully annealed primer compared to the primer having the 5' tail. Linear regression analysis of the data in Fig. 4C demonstrates that the percent primer displaced per unit of hel E was 2.5 fold greater for the fully annealed primer compared to the primer having the 5' tail. These results are consistent with the results in Fig. 4A demonstrating a 2.5 fold decrease in the level of displacement for primers having the 5' tail.

Hel E catalyzed displacement from gapped and nicked DNA substrates

DNA pol ϵ was purified based on the ability to complement repair deficient cells, suggesting a role in DNA repair (12). Our previous results demonstrating an interaction between hel E and pol ϵ suggest that hel E may also be involved in DNA repair (10). Therefore, we assessed the ability of hel E to act on substrates expected to be generated during DNA repair, *e.g.* double strand DNA with short gaps. The length of ss DNA required for hel E to bind to catalyze efficient strand displacement activity was examined. A 25 nucleotide long ss region 3' of the primer was effectively utilized by the helicase to support displacement of a downstream primer (data not shown). We have also demonstrated that hel E was capable of entering and displacing a DNA primer downstream of a 4 nucleotide gap (10). Fig. 5B shows the results of an experiment designed to determine whether hel E is capable of using a DNA substrate containing a one nucleotide gap upstream of the labeled primer. The results demonstrate that hel E can utilize the 1 nucleotide gap with only a minor decrease in activity compared to use of a 4 nucleotide gap.

An additional DNA substrate was designed to assess the ability of hel E to displace a primer from a nick in the DNA strand. The DNA substrate consisted of a 44 nucleotide template to which

Figure 6. RP-A stimulation of hel E. Reactions each contained 1 fmol of DNA substrate, M13mp183[32 P]18-mer, and increasing concentrations of calf thymus RP-A. Reactions were incubated at 37°C for 5 min. to allow RP-A to bind the DNA, and then were initiated with 0.04 units of hel E. Reactions were incubated for 20 min. at 37°C, stopped, and products analyzed as described in 'Materials and Methods'. Lane 1 is a heat denatured control; lane 2 is a no enzyme control. The concentration of RP-A is shown above the lanes.

the (–20) 17-mer universal sequencing primer and the (–47) 24-mer sequencing primer were annealed to the 5' and 3' portions of the template, respectively. Extending the primers with [α - 32 P]dCTP and dGTP resulted in a duplex DNA with a [32 P]-19-mer and [32 P]-25-mer generating a nick between the two primers. The results in Fig. 5C demonstrate that hel E is capable of entering at the nick in the DNA substrate and displacing a downstream primer, albeit with less efficiency compared to a DNA substrate containing a 4 nucleotide and 1 nucleotide gap. Hel E must be entering the DNA at the nick in the DNA substrate because it is unable to enter a fully duplex at the ends, as demonstrated in Fig. 5A.

Calf thymus RP-A stimulates displacement by hel E

Eukaryotic RP-A (single strand DNA binding protein) has been demonstrated to be involved in both DNA excision repair (13) and DNA replication (14). Therefore, we determined the effect calf thymus RP-A has on the displacement activity of hel E. Calf

thymus RP-A was purified to approximately 95% homogeneity as judged by silver staining of polyacrylamide gels. The RP-A had no detectable helicase activity (data not shown). The results presented in Fig. 6 demonstrate that RP-A stimulated the displacement activity of hel E. Quantitation of the results revealed a 2–3 fold increase in displacement by the addition of RP-A. This is similar to the level of stimulation by *E. coli* SSB previously reported for hel E (4). In addition, RP-A slightly stimulated the extent of displacement of larger DNA fragments, but had no effect on the size limitation of the displaced DNA fragment (data not shown). The mechanism of the stimulation of hel E by RP-A is currently under investigation. This phenomenon has also been observed for hel B, C, and D from calf (5).

DISCUSSION

Recently, there have been a number of DNA helicases identified in mammalian tissues. The majority of these enzymes have been characterized structurally with respect to size and subunit composition. Enzymologic characterization has also been performed. Based on these two criteria the helicases can be distinguished from one another. DNA hel E is a 100 kDa monomer that translocates in the 3' to 5' direction on the DNA strand to which it is bound, and preferentially hydrolyses ATP or dATP as sources of energy (4). Thömmes and Hübscher have identified four helicases in calf thymus, one of which has a 3' to 5' directionality, hel A (5). Hel A is thought to be a dimer of 47 kDa subunits, and is therefore structurally distinct from hel E. The other three helicases all translocate in the 5' to 3' direction, which distinguishes them from hel E. Two calf nuclear helicases also differ from hel E. NDH I is > 150 kDa and will utilize only ATP or dATP as energy sources, while NDH II is thought to be 100–130 kDa but uses all four NTP's and dNTP's as energy sources (15). Therefore, within calf, hel E is distinct from other reported helicases. RIP 100, a 100 kDa helicase isolated from human cells, may be the human homolog of hel E, but insufficient enzymological characterization exists to make a good comparison (16).

We have examined the activity of hel E on a variety of DNA substrates in order to elucidate its physiological role in DNA metabolism. Hel E is capable of displacing DNA fragments up to 140 nucleotides in length and is moderately processive for motion on ss DNA. This is in contrast to replicative prokaryotic helicases, which display processivities of motion on ss DNA on the order of several thousand. These helicases also display substantial inhibition of nucleoside triphosphate hydrolysis upon encountering the end of linear M13 DNA (17). In addition, these replicative helicases can displace DNA fragments in the range of tens of thousands nucleotides in length (18). The displacement length limitation of hel E is quite similar to that reported for hel B, C and D from calf thymus (5).

A 3' to 5' helicase involved in replication would be expected to track in front of the leading strand polymerase. A 5' tail on the displaced strand may then stimulate displacement by a replicative helicase. This effect has been observed with DNA hel δ from calf thymus (19). The finding that hel E is inhibited by such a structure is not consistent with a role for hel E in DNA replication. However, interaction with other proteins at the replication fork could be necessary if hel E is to participate in DNA replication.

While we can not rule out the possibility that hel E is involved in DNA replication, a series of experiments support a role in

DNA repair. Hel E can efficiently enter a DNA strand given a one nucleotide gap in the DNA substrate. In addition, hel E is capable of displacing a DNA primer given only a nick in the DNA substrate. This type of activity would be observed in excision repair following nicking of DNA on either side of a damaged DNA site, similar to that observed for DNA hel II from *E. coli* (20,21).

It was recently demonstrated that antibodies to RP-A inhibited excision repair from crude cell extracts, and that purified RP-A could overcome the inhibition (13). Consequently, the finding that RP-A stimulated the displacement activity of hel E is also consistent with a role for hel E in excision repair.

Following displacement of a DNA fragment, repair synthesis is necessary and evidence is available that pol α , β and ϵ all can participate in repair synthesis (12,22,23). Hel E shows partial co-migration with polymerase ϵ (4) and recent results have demonstrated that hel E and pol ϵ can functionally coordinate to carry out displacement synthesis (10). We therefore suggest that pol ϵ and hel E could participate in excision repair.

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