Escherichia coli Helicase II (uvrD Gene Product) Translocates Unidirectionally in a 3’ to 5’ Direction*

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The unwinding of duplex DNA to yield single-stranded template DNA for use by DNA polymerase is thought to be catalyzed by a class of enzymes called the helicases (1, 2). Helicases have been isolated from bacteriophage-infected cells, bacterial cells (for review see Ref. 1), and yeast (3). Helicases from bacteriophage T4 and phage T7 have been shown to have roles in DNA replication both as helicases and as part of the primase complex (4-6). The specific role(s) of the helicases isolated from bacterial cells and yeast is not yet known.

All helicases described to date are single-stranded DNA-dependent nucleoside 5’-triphosphatases (1). Presumably, nucleoside 5’-triphosphate (NTP) hydrolysis fuels translocation of the enzyme along the DNA molecule. This has been directly shown only for the bacteriophage T7 gene 4 protein (7) and as has been inferred for the other helicases. Several of the helicases have been shown to translocate unidirectionally along single-stranded DNA (6-12). The Escherichia coli rep protein translocates in a 3’ to 5’ direction with respect to the DNA strand on which it is bound. In this case, was assayed by the release of DNA susceptible to digestion by S1 nuclease. The rep protein of E. coli was shown to move in a 3’ to 5’ direction by the same method (8). Since the phage helicases, T7 gene 4 protein and T4 gene 41 protein, also participate in the synthesis of RNA primers on the lagging strand side of the replication fork, it is necessary that these enzymes translocate in a 5’ to 3’ direction (4-6, 9). Similarly, rep protein has a role in the replication of bacteriophage φX174 that suggests a 3’ to 5’ direction of translocation (14). Roles for the other helicases in E. coli are currently unknown, and therefore no obligatory direction of translocation is suggested by function for these enzymes. In this communication, a direct helicase assay has been used to determine the direction of translocation: for helicase II. This enzyme moves unidirectionally in a 3’ to 5’ direction with respect to the strand of DNA on which it is bound. This direction of translocation is opposite to that reported by Kuhn et al. (10, 11) and is consistent with a role for helicase II in excision repair (15, 16).

**EXPERIMENTAL PROCEDURES**

**Materials**

DNA and Nucleotides—Bacteriophage M13mp7 replicative form I DNA and single-stranded DNA were prepared as described (17). All unlabeled nucleotides were from P-L Biochemicals. [α-32P]dCTP and [γ-32P]ATP were from ICN Radiochemicals.

Enzymes—Restriction endonucleases, DNA polymerase I (large fragment), and bacteriophage T4 polynucleotide kinase were purchased from New England Biolabs; the reaction conditions used were those suggested by the supplier. E. coli helicase II was purified from

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E. coli harboring a multicopy plasmid carrying the structural gene for helicase II as described (18) with the following modifications. After harvesting, the cells (80 g) were resuspended in 50 mM Tris-HCl (pH 7.5), 10% sucrose, 10 mM EDTA and frozen in liquid nitrogen. The cells were lysed by the addition of lysozyme to 250 μg/ml and NaCl to 0.1 M followed by incubation at 0°C for 45 min. The cell suspension was heated in a 37°C water bath until the temperature reached 30°C and then transferred to an ice bath until the temperature reached 10°C. Lysed cells were centrifuged at 21,000 rpm for 60 min and the supernatant recovered as fraction I. Fraction II was applied to a single-harvesting, the cells (80 g) were resuspended in 50 mM Tris-HCl (pH 7.5), 10% glycerol, 0.1 mM EDTA, and 5 mM 2-mercaptoethanol (buffer A) containing 100 mM NaCl. Helicase II was eluted with buffer A containing 1 M NaCl. The enzyme was greater than 95% pure as judged by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and has a relative molecular mass of 76,000 g/mol. This is exactly the same size as helicase II purified by Abdel-Moneim et al. (19). Moreover, this preparation of helicase II has essentially the same apparent Km value for ATP and the same sensitivity to ATP analog inhibitors of the ATPase activity as previously described (19). In addition, this preparation of helicase II and purified rep protein does not migrate to the same position when directly compared on a polyacrylamide gel run in the presence of sodium dodecyl sulfate. By these criteria, this preparation of helicase II contains the same polypeptide as that used by Kuhn et al. (10, 11). Bacteriophage gene 41 protein and gene 61 protein were the generous gift of Dr. Nancy Nossal and Dr. Deborah Hinton (National Institutes of Health) (20, 21).

**Methods**

**Helicase Substrate Preparation.**—The DNA substrates used in helicase assays consist of the complementary strand of a radioactively labeled M13mp7 replicative form I HaeIII restriction fragment annealed to M13mp7 single-stranded DNA to form a partial duplex. The substrates were constructed by incubating the appropriate restriction fragment (approximately 100 ng) with M13mp7 single-stranded DNA (2 μg) in 40 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM dithiothreitol, 50 mM NaCl at 95°C for 5 min, followed by 65°C for 20 min, and then 23°C for 20 min. The resulting partial duplex was labeled at the 5'-OH of the restriction fragment in a reaction containing 5 units of DNA polymerase I (large fragment) and [α³²P]dCTP in the above buffer. Incubation was for 20 min at 23°C followed by the addition of 50 μl unlabeled dCTP and incubation at 23°C for an additional 20 min. After phenol extraction, the reaction mixture was passed through an agarose A-5m column (1.5 ml) equilibrated with 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 100 mM NaCl. The void volume containing the labeled DNA was collected and used directly as helicase substrate. It should be noted that this substrate may be contaminated with single-stranded DNA containing an unannealed restriction fragment.

The DNA substrate used to determine the direction of translocation of helicase II was constructed as above except that after annealing the complementary strand of the 341-base pair (bp) HaeIII restriction fragment with circular single-stranded M13mp7 DNA, the partial duplex was cleaved with ClaI and the single-stranded linear M13mp7 DNA. The enzyme was greater than 95% pure as judged by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and has a relative molecular mass of 76,000 g/mol. This is exactly the same size as helicase II purified by Abdel-Moneim et al. (19). Moreover, this preparation of helicase II has essentially the same apparent Km value for ATP and the same sensitivity to ATP analog inhibitors of the ATPase activity as previously described (19). In addition, this preparation of helicase II and purified rep protein does not migrate to the same position when directly compared on a polyacrylamide gel run in the presence of sodium dodecyl sulfate. By these criteria, this preparation of helicase II contains the same polypeptide as that used by Kuhn et al. (10, 11).

**RESULTS**

**E. coli** helicase II is a single-stranded DNA-dependent ATPase with helicase activity that translocates processively along single-stranded DNA (10, 11, 19). A processive mechanism for translocation suggests that translocation is unidirectional. Previous studies (10, 11) have suggested that helicase II translocates unidirectionally along single-stranded DNA in a 5’ to 3’ direction with respect to the strand on which it is bound. The question of the direction of translocation has been addressed in this report using a more direct helicase assay which measures the displacement of a labeled DNA fragment from a partial duplex DNA molecule.

**The Helicase Activity of Helicase II**—The helicase reaction catalyzed by helicase II has been demonstrated using an in vitro assay that measures the displacement of a labeled DNA fragment from a single-stranded circular DNA molecule to which the labeled fragment has been annealed. Three different partial duplex DNA molecules were tested as helicase substrates (Fig. 1). In Fig. 1, lanes 1–5, the DNA substrate used in the helicase reaction consists of a 71-nucleotide long, 3’-end-labeled DNA fragment annealed to M13mp7 single-stranded circular DNA. Lane 1, no enzyme; lane 2, 7 ng of helicase II; lane 3, 14 ng of helicase II; lane 4, 35 ng of helicase II; lane 5, 70 ng of helicase II. In lanes 6–10, the DNA substrate consists of a 3'-end-labeled 119-nucleotide DNA fragment annealed to M13mp7 single-stranded circular DNA. The 119-nucleotide long DNA fragment is frequently a doublet, probably due to the addition of two extra nucleotides onto the 3'-end of the annealed restriction fragment. This could occur if there was a small contamination of dTTP in the preparation of [α³²P]dCTP used in the labeling reactions. Lane 6, no enzyme; lane 7, 7 ng of helicase II; lane 8, 14 ng of helicase II; lane 9, 35 ng of helicase II; lane 10, 70 ng of helicase II. In lanes 11–15, the DNA substrate consists of a 3'-end-labeled 343-nucleotide DNA fragment annealed to single-stranded, circular M13mp7 DNA. Lane 11, no enzyme; lane 12, 7 ng of helicase II; lane 13, 14 ng of helicase II; lane 14, 35 ng of helicase II; lane 15, 70 ng of helicase II. All incubations were for 10 min at 37°C.

![](Image)

**Fig. 1. The helicase activity of helicase II.** Helicase activity was measured as detailed under “Experimental Procedures.” In lanes 1–5, the DNA substrate consists of a 3’-end-labeled 71-nucleotide fragment annealed to M13mp7 single-stranded circular DNA. Lane 1, no enzyme; lane 2, 7 ng of helicase II; lane 3, 14 ng of helicase II; lane 4, 35 ng of helicase II; lane 5, 70 ng of helicase II. In lanes 6–10, the DNA substrate consists of a 3’-end-labeled 119-nucleotide DNA fragment annealed to M13mp7 single-stranded circular DNA. The 119-nucleotide long DNA fragment is frequently a doublet, probably due to the addition of two extra nucleotides onto the 3'-end of the annealed restriction fragment. This could occur if there was a small contamination of dTTP in the preparation of [α³²P]dCTP used in the labeling reactions. Lane 6, no enzyme; lane 7, 7 ng of helicase II; lane 8, 14 ng of helicase II; lane 9, 35 ng of helicase II; lane 10, 70 ng of helicase II. In lanes 11–15, the DNA substrate consists of a 3’-end-labeled 343-nucleotide DNA fragment annealed to single-stranded, circular M13mp7 DNA. Lane 11, no enzyme; lane 12, 7 ng of helicase II; lane 13, 14 ng of helicase II; lane 14, 35 ng of helicase II; lane 15, 70 ng of helicase II. All incubations were for 10 min at 37°C.

**or by slicing the gel into 1-cm sections and counting in a liquid scintillation counter.**

**Other Methods—** Non-denaturing polyacrylamide gel electrophoresis was carried out as described (22). DNA concentrations were determined by directly measuring the absorbance at 260 nm and are expressed as nucleotide equivalents.

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1 The abbreviation used is: bp, base pairs.

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Asterisk was annealed to single-stranded, circular M13mp7 phage DNA. The reaction of helicase with the complementary strand of the 341-bp HaeIII restriction fragment of the linear DNA molecule is shown. In Fig. 1, lanes 6-10, the DNA fragment to the circular DNA molecule, the duplex region on the 3'-end of the 5'-end is 141 nucleotides in length. All available 3'-OH groups were labeled as described under “Experimental Procedures.” Asterisk denotes position of radioactive label.

Helicase II Translocates in a 3' to 5' Direction—To directly determine the direction of translocation of helicase II, the strategy depicted in Fig. 2 was adopted. Construction of the DNA substrate used in these experiments takes advantage of the presence of a single ClaI site within the duplex region of the 341-bp partial duplex DNA molecule. After annealing the DNA fragment to the circular DNA molecule, the duplex region was cleaved with ClaI. Cleavage with ClaI generates three 3'-OH groups that can be labeled using DNA polymerase I (large fragment) and the appropriate [α-32P]dNTPs, one on the 200 nucleotide fragment, one on the 141 nucleotide fragment, and one on the linear M13mp7 DNA molecule. After labeling all available 3'-OH groups, the DNA substrate shown in Fig. 2 was used in helicase reactions catalyzed by helicase II (Fig. 3). Helicase II catalyzes the displacement of the 143-nucleotide long fragment from this linear DNA substrate but fails to catalyze the displacement of the 202-nucleotide long fragment (Fig. 3, lanes 2 and 3). When ATP is omitted from the reaction mixture, there is no displacement of the fragment (Fig. 3, lane 4), confirming the ATP dependence of the reaction. As a control, the 343-bp partial duplex circular DNA substrate was used as a helicase substrate under the same conditions (Fig. 3, lane 7). As expected, helicase II catalyzes the displacement of the 343-nucleotide long fragment from the circular DNA molecule. These data suggest that helicase II translocates unidirectionally, but in the 3' to 5' direction.

Results obtained previously by others (10, 11) have suggested that helicase II translocates in a 5' to 3' direction, opposite to the direction of translocation reported here. Translocation in the 5' to 3' direction is predicted to cause the displacement of the 3'-end of a DNA fragment first. As a control for the experiments presented above, this prediction was tested using the linear helicase substrates shown in Fig. 4A. The construction of these DNA substrates takes advantage of restriction sites located within the duplex DNA of the 343-bp partial duplex DNA substrate. There is a ClaI site located 202 nucleotides from the 3'-end of the annealed [32P]DNA fragment and a RsaI site located 163 nucleotides from the 3'-end of the annealed [32P]DNA fragment (Fig. 4A). The linear helicase substrates shown were constructed by incubating 343-bp partial duplex DNA (after labeling the 3'-end) with the appropriate restriction endonuclease. The resulting DNA substrate is a linear molecule with a labeled DNA fragment of discreet length annealed to one end. A second, unlabeled DNA fragment is annealed at the opposite end of the linear M13mp7 DNA molecule.

An enzyme that translocates unidirectionally in the 5' to 3' direction should displace the labeled DNA fragment from the 3'-end. The HaeIII fragment isolated after digestion of M13mp7 replicative form I DNA is 341 base pairs in length. After the 3'-end labeling reaction in the presence of [α-32P]dCTP, the fragment is 343 base pairs in length.

3J. W. George and S. W. Matson, manuscript in preparation.

4The HaeIII fragment isolated after digestion of M13mp7 replicative form I DNA is 341 base pairs in length. After the 3'-end labeling reaction in the presence of [α-32P]dCTP, the fragment is 343 base pairs in length.
the linear substrates shown in Fig. 4A. Each linear DNA substrate was incubated with helicase II under conditions that are optimal for the helicase reaction. Displacement of the labeled DNA fragment from the linear substrate catalyzed by helicase II was not observed for either of the DNA substrates tested (Fig. 4B, lanes 6 and 10), although the full-length 343-nucleotide long fragment was displaced from the single-stranded circular substrate under these conditions (Fig. 4B, lane 2). As a positive control for this experiment, the bacteriophage T4 gene 41 protein-gene 61 protein complex was used in a helicase reaction (Fig. 4B, lanes 3, 7, and 11). The gene 41 protein-gene 61 protein complex is a helicase/primease that has been shown to move in a 5' to 3' direction along single-stranded DNA (6). As expected, the 3'-end-labeled DNA fragments were displaced from the linear substrates and the 343-nucleotide DNA fragment was displaced from the single-stranded circular substrate (Fig. 4B, lanes 3, 7, and 11). This confirms the direction of translocation of the gene 41 protein-gene 61 protein complex and further suggests that helicase II does not move in the 5' to 3' direction but in the 3' to 5' direction.

**Fig. 4. Helicase II and the phage T4 gene 41-gene 61 complex translocate in opposite directions.** Panel A, the DNA substrates used in the helicase reaction were constructed by cleaving the 3' end-labeled 343-bp partial duplex DNA substrate with either Clal or Rsal prior to use in a helicase reaction. The resulting DNA substrates are linear molecules with a labeled DNA fragment of discreet length annealed at the 3'-end of the linear M13 phage DNA. There is also a DNA fragment annealed at the 5'-end of the linear phage DNA molecule; this DNA fragment is not labeled. Panel B, helicase reactions were as described under "Experimental Procedures." In lanes 1–4, the DNA substrate consists of a 3'-end-labeled 343-bp DNA fragment annealed to circular M13mp7 DNA. Lane 1, no enzyme; lane 2, 35 ng of helicase II; lane 3, 1.7 µg of bacteriophage T4 gene 41 protein and 0.1 µg of phage T4 gene 61 protein; lane 4, heat-denatured control (100 °C for 3 min). In lanes 5–8, the DNA substrate is the linear molecule resulting from cleavage with Clal as shown in panel A. The labeled fragment is 202 nucleotides long. Lane 5, no enzyme; lane 6, 35 ng of helicase II; lane 7, 1.7 µg of phage T4 gene 41 protein and 0.1 µg of phage T4 gene 61 protein; lane 8, heat-denatured control. In lanes 9–12, the DNA substrate is the linear DNA molecule resulting from cleavage with Rsal as shown in panel A. The labeled fragment is 163 nucleotides long. Lane 9, no enzyme; lane 10, 35 ng of helicase II; lane 11, 1.7 µg of phage T4 gene 41 protein and 0.1 µg of phage T4 gene 61 protein; lane 12, heat-denatured control. Asterisk denotes position of radioactive label.

Reciprocal experiments were performed in which a 341-bp partial duplex DNA substrate was labeled at the 5'-end using bacteriophage T4 polynucleotide kinase prior to incubation with the appropriate restriction endonuclease (Fig. 5). The linear DNA substrates obtained after restriction digestion contain a single labeled DNA fragment of discreet length but, in this case, the labeled DNA fragment is on the 5'-end of the linear DNA molecule (refer to Fig. 4A). As suggested by the results presented above, the 5'-end-labeled fragment was displaced by helicase II using this DNA substrate (Fig. 5, lanes 2 and 5). Taken together, these results strongly suggest that helicase II translocates in the 3' to 5' direction along single-stranded DNA.

In addition to the experiments presented above, a kinetic analysis of the displacement of a DNA fragment from a linear molecule has been carried out using the DNA substrates shown in Fig. 6. In each case, a 3'-end-labeled circular DNA substrate has been converted into a linear DNA substrate by cleaving within the single-stranded region of the circular DNA molecule using EcoRI.5 One DNA substrate has a single-stranded 3'-tail that is 817 nucleotides long and a duplex region of 71 bp, and the other DNA substrate has a single-stranded 3'-tail that is approximately 7000 nucleotides long and a duplex region of 119 bp. Each DNA substrate was incubated with helicase II, and the amount of fragment displaced from the linear DNA substrate has been compared with the amount of fragment displaced from a circular sub-
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FIG. 6. DNA substrates for a kinetic analysis of the direction of translocation. The 71-bp partial duplex DNA substrate and the 119-bp partial duplex DNA substrates were constructed and labeled as described under “Experimental Procedures.” Linear DNA substrates were obtained by incubating the circular DNA substrate with the restriction endonuclease EcoRI which can cleave at the hairpin present in M13mp7 phage DNA. The resulting 3' single-stranded regions are 817 nucleotides and about 7000 nucleotides in length. Asterisk denotes position of radioactive label.

TABLE I

A kinetic analysis of the direction of translocation of helicase II

Reaction mixtures were as described under “Experimental Procedures” using the indicated amounts of E. coli helicase II. Incubations were for 20 min at 37°C. The amount of labeled fragment displaced was determined by slicing the polyacrylamide gel into 1-cm sections and counting in a liquid scintillation counter.

| Helicase substrate                  | Fragment displaced | Helicase II | Comparison ratio |
|-------------------------------------|-------------------|-------------|-----------------|
| 817-base 3'-tail linear substrate  | %                 | 9g          |                 |
| Circular substrate                  | 25.9              | 17          | 0.88            |
| 7000-base 3'-tail linear substrate | 38.0              | 17          | 0.88            |
| Circular substrate                  | 48.8              | 17          | 1.41            |
| 817-base 3'-tail linear substrate  | 34.4              | 17          | 0.73            |
| Circular substrate                  | 41.3              | 34          |                 |
| 7000-base 3'-tail linear substrate | 56.6              | 34          | 1.17            |
| Circular substrate                  | 69.6              | 34          |                 |
| 7000-base 3'-tail linear substrate | 59.3              | 34          |                 |

strate with the same length of duplex DNA (Table I).

If helicase II translocates in a 3' to 5' direction, the linear DNA substrate with a short single-stranded 3'-tail is expected to be a relatively poor helicase substrate compared to a circular DNA molecule. This is expected because the long 5'-tail will bind enzyme molecules more frequently than the short 3'-tail, since it is 8-fold longer, allowing 3' to 5' translocation but no displacement reaction. Only when the enzyme binds the relatively short 3'-tail will it encounter the labeled DNA fragment after 3' to 5' translocation and cause a displacement reaction. This is in contrast to the situation encountered on a circular DNA molecule where binding at any site and unidirectional translocation will ultimately bring the enzyme to duplex DNA. Conversely, the linear DNA substrate with the long single-stranded 3'-tail should allow binding with subsequent 3' to 5' translocation resulting in a displacement reaction almost as frequently as circular DNA substrate.

The results presented in Table I confirm these predictions. Comparison of the fraction of labeled DNA fragment displaced from the linear DNA substrate with a short 3'-tail and the corresponding circular substrate shows that a displacement reaction occurs on the linear molecule less frequently than on the circular molecule. However, when the labeled DNA fragment is moved to the opposite end of the linear molecule, creating a long single-stranded 3'-tail, a displacement reaction is catalyzed by helicase II more frequently on the linear substrate than observed using the corresponding circular DNA substrate. These results are consistent with unidirectional translocation in the 3' to 5' direction. The displacement of more labeled DNA fragment from the linear substrate with a long single-stranded 3'-tail as compared to the circular DNA substrate reflects a processive mechanism of translocation along single-stranded DNA (see “Discussion”).

DISCUSSION

Many enzymes that interact with DNA do so with a specific polarity. This is true for the DNA polymerases and the RNA polymerases that synthesize nucleotide chains in the 5' to 3' direction, for a number of exonucleases which degrade DNA in a specific direction, and for many other enzymes (23). The helicases, which catalyze the unwinding of duplex DNA, also exhibit a directionality in their interaction with DNA. Each individual helicase translocates in either the 5' to 3' direction or the 3' to 5' direction with respect to the DNA strand on which it is bound. In this context, the E. coli helicases I and III unwind DNA in the 5' to 3' direction (10-12).

In this paper, it has been demonstrated that helicase II translocates along single-stranded DNA in the 3' to 5' direction. This direction of translocation is opposite to that reported previously (10, 11). The conclusions reported here are based on direct observation of the end products of a helicase II reaction using polyacrylamide gels to resolve reaction products. In the experimental protocol used (Fig. 2), helicase II is provided with a DNA substrate on which it should be able to translocate in either the 5' to 3' direction or the 3' to 5' direction to reach duplex DNA. The helicase reaction is followed by observing the labeled DNA fragments that are displaced from the linear partial duplex DNA substrate by helicase II. The results suggest that helicase II migrates along single-stranded DNA in a 3' to 5' direction and displaces the DNA fragment on the 5'-end of the linear substrate molecule while failing to displace the DNA fragment on the 3'-end of the linear substrate molecule. This interpretation is based on two facts. First, helicase II translocates along single-stranded DNA processively² and therefore in a single direction. Second, helicase II requires single-stranded DNA for binding (10); this condition is not met at either end of the linear DNA molecule shown in Fig. 2. Therefore, helicase II will bind to the interior, single-stranded region and translocate in one direction, ultimately displacing a labeled DNA fragment of specific length. The results shown in Fig. 3 clearly demonstrate translocation in the 3' to 5' direction.

In addition to the direct analysis of a helicase reaction, a kinetic analysis of the displacement of DNA fragments from linear DNA molecules was consistent with 3' to 5' translocation. In one case, the linear partial duplex substrate had a DNA fragment annealed at a position 817 nucleotides from the 3'-end of the linear DNA molecule. Compared with the length of single-stranded DNA on the other side of the duplex region (approximately 6300 nucleotides), this target for initial binding of helicase II is small. Therefore, only a small percentage of enzyme molecules will bind and initiate 3' to 5' translocation into a duplex region of DNA. The remainder will bind and translocate to the end of the DNA molecule without encountering the duplex region of the substrate. The results obtained in comparing displacement of the fragment from the linear substrate versus the circular substrate were

- **Helicase substrate**
  - Fragment displaced
  - Helicase II
    - Comparison ratio

- **Comparison ratio**
  - Linear %
  - Circular %

- **Helicase II**
  - Fragment displaced
  - Comparison ratio

- **Comparison ratio**
  - Linear %
  - Circular %
consistent with this idea. Even at high helicase II concentrations, less labeled DNA fragment was displaced from the linear molecule with a short 3'-tail than from the circular molecule. When the DNA fragment is annealed on the 5'-end of the linear DNA molecule, the target for initial binding on the 3' side of the duplex region is large. Consequently, most of the enzyme molecules will bind in this region of single-stranded DNA and, if translocation is in the 3' to 5' direction, translocate toward duplex DNA. On this linear DNA substrate, helicase II catalyzed the displacement of nearly 40% more labeled DNA fragment than when a circular DNA substrate was used. Two conclusions can be drawn from this result. First, the initial binding rate must be nearly as large on this linear substrate molecule as it is on the circular molecule. Second, the unidirectional translocation is processive. A processive mechanism for translocation suggests that helicase II will remain bound on the circular DNA substrate after displacing the labeled DNA fragment and continue unidirectional translocation. On the linear DNA substrate, an end will necessarily be reached and the enzyme will dissociate and bind a new substrate molecule. Since the new substrate molecule will be a partial duplex, another displacement event will be catalyzed. The result will be a greater displacement of labeled DNA fragments from the linear substrate than from the circular substrate during the initial stages of the reaction. This is consistent with results we have obtained in the analysis of the helicase II ATPase reaction. Although the kinetic results only suggest that helicase II may translocate in the 3' to 5' direction, coupled with the direct analysis of translocation direction, it is clear that helicase II translocates in a 3' to 5' direction along the DNA strand on which it is bound.

The results presented here indicate a direction of translocation that is opposite to that reported by others (10, 11). Previous conclusions relied on an indirect assay of the helicase reaction catalyzed by helicase II. In those experiments, a linear duplex DNA molecule was incubated with either exonuclease III or λ exonuclease prior to incubation with helicase II. This provided a single-stranded DNA binding site for helicase II with a 5' polarity or a 3' polarity. The DNA substrates were subsequently incubated with helicase II, and the amount of single-stranded DNA formed was determined after digestion with S1 nuclease. The results suggested that a suitable substrate for helicase II was generated when the DNA substrate was partially degraded with exonuclease III but not with λ exonuclease. Since exonuclease III digests DNA in a 3' to 5' direction, the single-stranded DNA tail remaining on the duplex would have the correct polarity for an enzyme that translocates in the 5' to 3' direction. It was inferred from these experiments that helicase II translocated in the 5' to 3' direction. This conclusion was apparently confirmed by experiments utilizing substrates similar to the ones used in this report. However, the DNA fragments displaced by the helicase were analyzed on sucrose gradients rather than polyacrylamide gels. Subsequent hybridization analysis confirmed the identity of the fragment displaced, which was consistent with 5' to 3' translocation. Presently, I have no explanation for the discrepancy in results. The experiments reported here are more direct and of higher resolution than those reported previously.

The direction of translocation of several prokaryotic helicases is suggested by their function in the cell (4-6, 9, 14). Translocation in the 3' to 5' direction is consistent with a role for helicase II in excision repair mediated by the uvrABC incision nuclease (15, 16). Although 5' to 3' translocation is also possible. A model for helicase II interaction with DNA in such a repair scheme is shown in Fig. 7. In this model, helicase II could interact with DNA polymerase I and/or the uvrABC enzyme complex and move along the template strand in the 3' to 5' direction. Such an interaction with DNA polymerase I has been suggested (15). Translocation of helicase II through the duplex could facilitate displacement of the fragment that has been incised by the uvrABC nuclease allowing that enzyme to turn over and providing DNA template for DNA polymerase I. In this model, translocation in a 3' to 5' direction is consistent with an interaction with DNA polymerase I and it maintains helicase II on the template DNA rather than bound to the fragment being displaced. Many aspects of this model remain to be elucidated and clarified. For instance, is there an interaction between uvrABC and helicase II? How does helicase II bind the nicked DNA-protein complex and how does it dissociate to leave a nick for ligase to seal? These questions await further experimental testing.

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