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Gene 4 helicase of bacteriophage T7 mediates strand transfer through pyrimidine dimers, mismatches, and nonhomologous regions

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ABSTRACT **In bacteriophage T7 the gene *2.5* single-stranded DNA-binding protein and the gene *4* helicase together promote the annealing of homologous regions of two DNA partners to form a joint molecule and subsequent strand transfer. In this reaction T7 gene *2.5* protein is essential for joint molecule formation, but is not required for T7 gene *4* protein-mediated strand transfer. T7 gene *4* helicase alone is able to mediate strand transfer, provided that a joint molecule is available. The present paper shows that, in addition, strand transfer proceeds at a normal rate even when both DNA partners contain ultraviolet-induced pyrimidine dimers (0.6 dimer per 100 nt). An insert of a relatively long (842-nt) segment of nonhomologous DNA in the single-stranded DNA partner has no effect on strand transfer, whereas its presence in the double-stranded partner prevents strand transfer. A short insert (37 nt) can be tolerated in either partner. Thus, DNA helicase is able to participate in recombinational DNA repair through its role in strand exchange, providing a pathway distinct from nucleotide excision repair.**

Recent studies have shown that the gene *41* DNA helicase encoded by bacteriophage T4 (1) and the gene *4* helicase encoded by bacteriophage T7 (2) mediate homologous DNA strand transfer within homologous DNA molecules. The rate and polarity of strand transfer are in accord with the properties of these enzymes in catalyzing strand displacement at a replication fork. A convenient DNA substrate for dissecting the strand transfer reaction is a joint DNA molecule formed between a single-stranded circle and a homologous linear duplex DNA molecule with a short 3′-single-stranded tail (Fig. 1). In the preparation of this joint molecule we have used the T7 gene *2.5* ssDNA-binding protein to mediate the efficient annealing of the two partner molecules (2, 3). As depicted in Fig. 1, strand transfer driven by the helicases is coupled to the hydrolysis of a nucleoside 5′-triphosphate and proceeds in a polar 5′-to-3′ direction with respect to the invading strand (2, 3).

In the studies cited above (2, 3) the DNA sequences of the two DNA molecules were identical. However, in homologous genetic recombination *in vivo* the two partners are derived from different parents and thus may differ in nucleotide sequence. Consequently, for the helicase-mediated strand exchange to function in homologous recombination it must be capable of forming heteroduplex DNA molecules containing mismatched base pairs as well as insertions and deletions, thus providing a source of genetic variability. Homologous recombination constitutes one of three DNA repair pathways and thus plays an important role in the survival of organisms when their genomic DNA is damaged by UV light or by other physical and chemical agents (4). The need for recombinational repair arises when DNA synthesis resumes distal to a lesion that has blocked the progress of the replication process and thus leaves a lesion not accessible to excision repair, for instance, thymine dimers within a single-stranded gap (5–7).

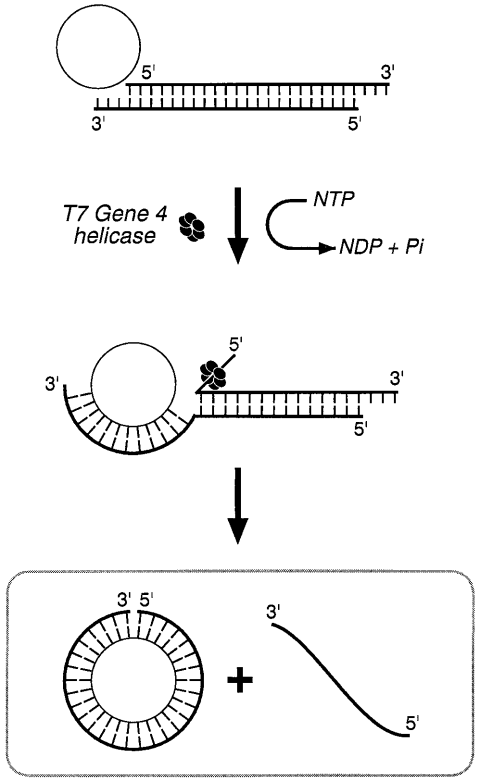

FIG. 1. Scheme for T7 gene *4* helicase-mediated strand transfer. A joint molecule consisting of circular, single-stranded M13 DNA annealed to a 3′-single-stranded terminus of homologous linear, double-stranded M13 DNA. The hexameric T7 gene *4* helicase binds to the 5′-displaced single-stranded terminus created by spontaneous branch migration and translocates in a 5′-to-3′ direction to unwind the duplex DNA at the expense of the hydrolysis of a NTP. As the unwinding proceeds the newly exposed single-stranded DNA (ssDNA) on the duplex partner base pairs to the complementary region on the ssDNA circle. The continuous polar strand transfer results in the formation of a circular, double-stranded DNA (dsDNA) molecule and a linear, ssDNA molecule.

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Abbreviations: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; SSB protein, ssDNA-binding protein; RF1, replicative form 1.

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The RecA protein, the major recombination protein of *Escherichia coli*, has been shown to be involved in recombinational repair. Purified RecA protein mediates the formation of heteroduplex DNA that contains base pair mismatches as well as insertions that are hundreds of nucleotides long (8). In addition, the RecA protein can mediate strand exchange between circular ssDNA containing pyrimidine dimers and a homologous linear duplex DNA (9), thus accounting for its known role *in vivo* in recombinational repair of UV damage (10–13).

Recombination (14) and DNA repair (15, 16) in *E. coli* cells infected with bacteriophage T7 are extremely efficient, and recombination is not impaired by the absence of the host RecA pathway (17). Consequently, we believe that the T7 gene *4* helicase-mediated strand exchange reaction provides the major pathway for recombination in *E. coli* cells infected with bacteriophage T7 (2). In the present study we have examined the ability of the T7 gene *4* helicase to participate in strand transfer reactions to produce heteroduplex molecules containing pyrimidine dimers, base pair mismatches, and insertions in each of the two DNA strands similar to those described above for the *E. coli* RecA protein.

An additional incentive for these studies has come from recent evidence that defects in DNA helicase play an important role in a number of human diseases thought to arise from defects in DNA repair processes. For example, genetic defects in putative DNA helicases have been proposed to account for xeroderma pigmentosum, Cockayne syndrome, Bloom syndrome, and Werner syndrome (18–21). With the exception of xeroderma pigmentosum, it remains to be determined if the defect reflects a defect in helicase-mediated strand exchange, in DNA replication, or in the excision-repair pathway. In this communication we show that T7 helicase-mediated strand exchange can provide a mechanism for DNA repair, a reaction that becomes more important in those cells that do not have a RecA type enzyme.

MATERIALS AND METHODS

Proteins and DNA. The 63-kDa T7 gene *4* protein, a species of gene *4* protein that has both helicase and primase activities, was overexpressed and purified to apparent homogeneity (>98% pure) from *E. coli* cells by B. Beauchamp (Harvard Medical School) as described (22). T7 gene *2.5* protein was purified to apparent homogeneity (>98% pure) as previously described (2). T7 gene *6* protein (5′-to-3′ exonuclease), *E. coli* single-stranded DNA-binding protein (SSB protein) and restriction enzymes were purchased from Amersham. M13, M13mp18, M13mp18-Δ37, and fd replicative form 1 (RF1) DNA and circular ssDNA were prepared according to the methods described (23, 24). M13mp18-Δ37, which contains a deletion of 37 bp between restriction enzyme sites *Sac*I and *Pst*I, is derived from plasmid M13mp18. M13mp18-Δ37 was constructed by first digesting M13mp18 RF1 DNA with *Sac*I and *Pst*I, removing the terminal overhang with mung bean nuclease, and then forming a circular DNA with T4 DNA ligase.

Preparation of Joint DNA Molecules with T7 Gene *2.5* Protein. To prepare linear dsDNA having 3′-termini, restriction enzyme *Alw*NI and T7 gene *6* protein, a 5′-to-3′ exonuclease, were used except that *Hpa*I enzyme was used to linearize the double-stranded fd DNA used in the experiment described in Fig. 2. Joint DNA molecules consisting of circular ssDNA annealed to the single-stranded region of homologous linear dsDNA having 3′ single-stranded termini were prepared by incubating circular, single-stranded M13, fd, M13mp18, or M13mp18-Δ37 DNA (10 μM nucleotide equivalent) and linear, duplex M13, fd, M13mp18, or M13mp18-Δ37 DNA having single-stranded termini (20 μM) in a standard reaction mixture containing 25 mM Tris·HCl (pH 7.5), 5 mM dithiothreitol (DTT), 10 mM $MgCl_2$, and 2 μM T7 gene *2.5* protein (2, 3). After incubation at 32°C for 20 min, the reaction was stopped and the gene *2.5* protein was removed by extraction with phenol as previously described (3). Agarose gel electrophoresis confirmed that 40–80% of the DNA substrates were present as joint molecules.

Strand Transfer Mediated by T7 Gene *4* Protein. The strand transfer reaction mixture contained 25 mM Tris·HCl (pH 7.5), 5 mM DTT, 15 mM $MgCl_2$, 5 mM dTTP, 10 μM joint molecule, and 0.2 μM gene *4* protein. The mixture was incubated at 32°C for 20 min and the reaction was stopped by the addition of EDTA (50 mM), SDS (0.5%), and proteinase K (0.6 mg/ml). After an additional incubation at 32°C for 10 min, samples were analyzed by electrophoresis through 0.8% agarose gel in TAE buffer (0.04 M Tris–acetate/0.001 M EDTA, pH 8.0) at 0.5 V/cm for 15 hr. After electrophoresis, the gel was stained in TAE buffer containing 0.5 μg/ml ethidium bromide and the DNA bands were illuminated by UV light and photographed.

Irradiation of Joint DNA Molecule. Pyrimidine dimers in the joint DNA molecules were formed by UV irradiation. The joint molecules (10 μl at a concentration of 10 μM) in TE buffer (10 mM Tris/1 mM EDTA, pH 7.5) were irradiated with light from a germicidal UV lamp. The dose of UV was 48 $J \cdot m^{-2} \cdot sec^{-1}$. The joint DNA molecules were irradiated for 25, 50, 100, and 200 sec, and aliquots were removed at each time. The joint molecules that received 25 and 50 sec of UV irradiation had approximately 130 and 260 pyrimidine dimers, respectively, based on 1 pyrimidine dimer per 1000 bp of DNA created by a fluence of 100 J/m^2 (25). This calculation is in agreement with data of Livneh and Lehman (9)

Visualization of Products of the Strand Exchange Reaction. Products of the strand transfer reaction were examined by electron microscopy. Strand transfer reaction mixtures were incubated at 32°C for 1 min or 20 min and deproteinized by extraction with phenol as described above. The DNA samples were incubated with *E. coli* SSB protein (2 μg/ml) in a solution of 20 mM Hepes, pH 7.5/1 mM EDTA at 20°C for 5 min. SSB protein specifically binds to the ssDNA. After incubation, the DNA–protein complex was fixed by incubation with 0.6% glutaraldehyde for an additional 5 min at room temperature. The free protein and fixatives were removed by gel filtration through a 2-ml column of Bio-Gel A5M. The DNA samples were adsorbed to thin carbon foils, washed, air dried, and subjected to rotary shadowcasting with tungsten as described (26).

RESULTS

Helicase-Mediated Formation of Heteroduplex DNA Containing Mismatched Base Pairs. T7 gene *4* helicase mediates strand transfer between two completely homologous DNA molecules present in a joint molecules such as that depicted schematically in Fig. 1 (2, 3). Strand transfer proceeds in a polar 5′-to-3′ direction with respect to the invading strand at a rate of at least 120 nt/sec (2). To examine the possible effect of differences in nucleotide sequence between the two participating DNA molecules on the extent or rate of strand transfer we used T7 gene *2.5* protein to prepare joint molecules consisting of circular single-stranded M13 DNA and linear duplex fd DNA. These two relatively homologous DNAs, whose nucleotide sequences differ by 3% (27), were previously used by Bianchi and Radding (8) to examine the ability of the *E. coli* RecA protein to mediate the formation of DNA heteroduplexes with mismatched base pairs.

As shown in Fig. 2, lane 4, T7 gene *4* helicase mediates the complete transfer of one strand from a full-length linear duplex fd DNA to the circular single-stranded M13 DNA molecule. The formation of a circular duplex DNA molecule was demonstrated by agarose gel electrophoresis. The amount

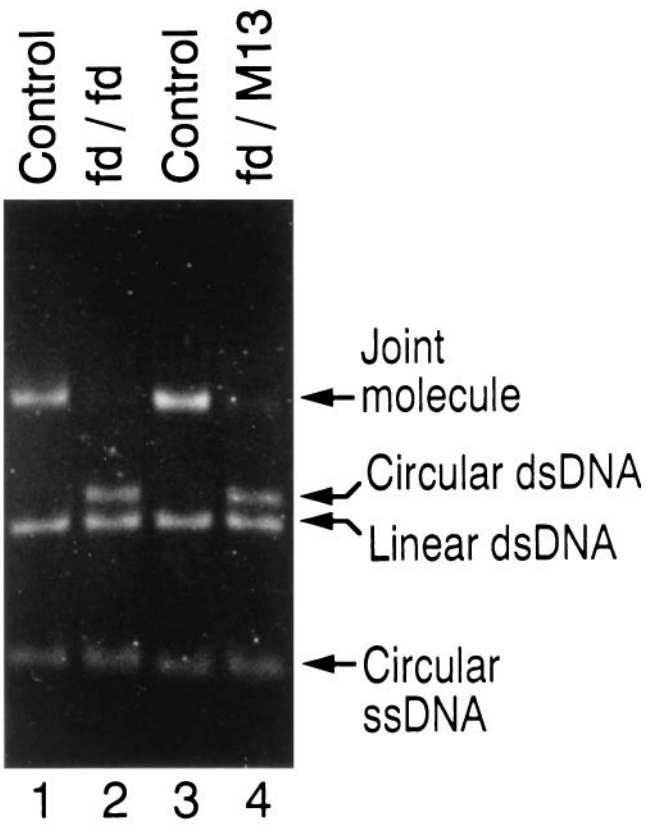

FIG. 2. Strand transfer between fd and M13 DNA molecules. Joint DNA molecules composed of circular single-stranded M13 or fd DNA annealed to the 3′-single-stranded termini of linear double-stranded fd DNA were prepared with T7 gene *2.5* protein and subsequently deproteinized by extraction with phenol/chloroform. Strand transfer reactions were carried out as described in *Materials and Methods*, and reaction samples were analyzed by agarose gel electrophoresis. Lanes 1 and 3, joint molecules as controls; lane 2, strand transfer between circular single-stranded fd and linear double-stranded fd DNA molecules; lane 4, strand transfer between circular single-stranded M13 and linear double-stranded fd DNA molecules. Positions of various DNA species are indicated.

of product after 20 min of incubation was identical to that obtained when the joint molecule consisted of completely homologous fd DNA (Fig. 2, lane 2). We also analyzed the products of strand transfer at 1, 5, and 10 min and found no difference in the extent of strand transfer between the M13/fd joint molecules and the fd/fd joint molecules, thus suggesting that the rate of strand exchange is not significantly affected by a 3% difference in nucleotide sequence between the two DNA molecules (data not shown).

Strand Exchange Between DNA Molecules Containing Pyrimidine Dimers. Cells exposed to UV light, a strong mutagenic agent, acquire DNA damage in the form of pyrimidine dimers. In *E. coli* these lesions can be removed either by photoreactivation or through the excision-repair pathway (28, 29). In the event that the pyrimidine dimers are not removed the DNA polymerase either polymerizes nucleotides through the lesion in a non-template-mediated reaction or it ceases synthesis at these sites and resumes nucleotide polymerization downstream (30). In the latter case the newly synthesized DNA contains single-stranded gaps with an average size of 1000 nt, a region that is now susceptible to endonucleolytic attack. This postreplication gap can be eliminated through a recombination event in which the complementary segment from the sister duplex molecule fills in the gap (31). In *E. coli* the RecA protein is known to participate in such recombination repair (32, 33). Livneh and Lehman (9) have shown that purified RecA protein can promote the exchange of DNA strands between ssDNA containing pyrimidine dimers and a homologous duplex DNA. However, about 20 sec is required for branch migration to pass a pyrimidine dimer, a rate that is about 1/50 of that obtained with ssDNA lacking these lesions.

Since the strand transfer reaction mediated by the T7 helicase has the potential to participate in postreplication recombinational repair in a manner analogous to the RecA protein, we have examined the ability of the T7 gene *4* protein to catalyze strand transfer between DNA molecules containing pyrimidine dimers. In the experiment shown in Fig. 3 joint molecules were prepared and then subjected to various doses of UV irradiation to obtain preparations of joint molecules that contained 130–260 pyrimidine dimers. The presence of 130–260 pyrimidine dimers (Fig. 3, lanes 2 and 3) had little effect on the ability of the gene *4* helicase either to translocate through the lesions or to catalyze strand transfer. Longer periods of UV irradiation appeared to destroy a portion of the joint molecules, but strand transfer took place nonetheless (Fig. 3, lanes 4 and 5). We did not detect a difference in the rate of strand transfer using a joint molecule containing 130 pyrimidine dimers compared with strand transfer on a joint molecule lacking these lesions when shorter times were examined (data not shown). These results show that the T7 helicase mediates strand transfer efficiently even when the number of pyrimidine dimers is as high as approximately 0.6/100 nt.

T7 Helicase Promotes Branch Migration Through Nonhomologous DNA Regions. To examine the effect of nonhomologous DNA regions in either the ssDNA or dsDNA partner on strand transfer we prepared joint molecules in which each partner had a segment of DNA that is absent from the other partner. Joint molecules in which each DNA has a relatively short insert were constructed by annealing circular single-stranded M13mp18 DNA to a linear duplex M13mp18-Δ37 DNA through a 3′-single-stranded terminus. M13mp18-Δ37 is lacking a 37-nt segment found in M13mp18. Joint molecules were prepared in which the circular ssDNA contained the

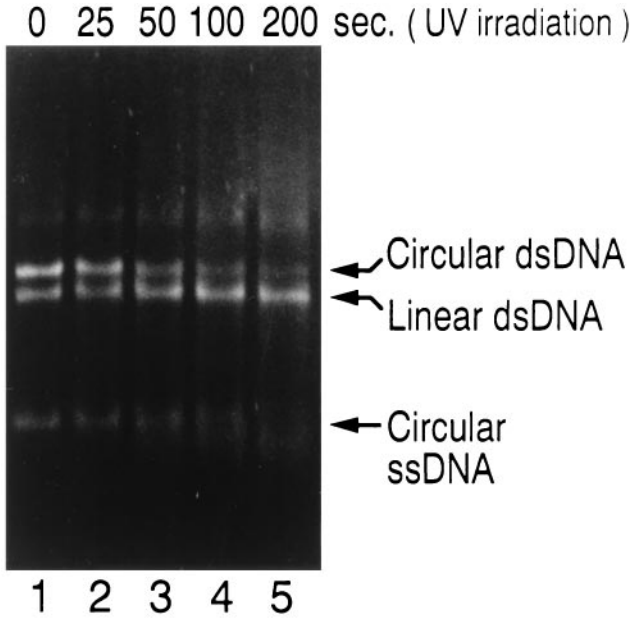

FIG. 3. Strand transfer between UV-irradiated DNA molecules. Joint DNA molecules composed of circular single-stranded M13mp18 and linear double-stranded M13mp18 DNA molecules were formed with T7 gene *2.5* protein and subsequently deproteinized by extraction with phenol/chloroform. The joint molecules were irradiated with UV light 25–200 sec to produce 130–260 pyrimidine dimers per joint molecule. Strand transfer reactions were carried out as described in the legend to Fig. 2. The products were analyzed by agarose gel electrophoresis and a photograph of the gel is presented.

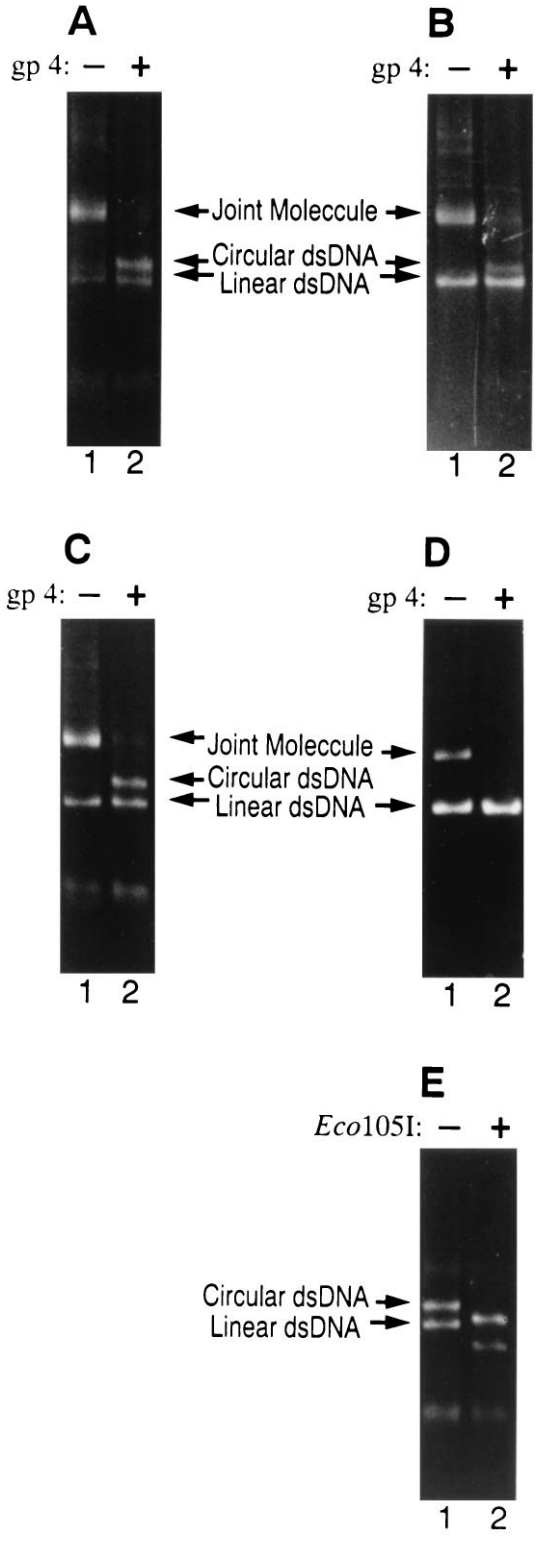

FIG. 4. Effect of nonhomologous regions on T7 gene *4* helicase-mediated strand transfer. To prepare joint molecules in which one of the two partners contains nonhomologous regions of DNA we used M13, M13mp18, and M13mp18-Δ37 DNA. M13mp18 DNA contains an insert of 842 nt compared with M13 DNA. M13mp18-Δ37 has a deletion of 37 nt compared with M13mp18 DNA. Joint molecule formation, strand transfer reactions, and agarose gel electrophoresis were performed as described in the text. The presence (+) or absence (−) of gene *4* protein is indicated. (*A*) Strand transfer between circular ssM13mp18 and linear dsM13mp18-Δ37 DNA molecules. (*B*) Strand transfer between circular ssM13mp18-Δ37 and linear dsM13mp18 DNA. (*C*) Strand transfer between circular ssM13mp18 and linear dsM13 DNA. (*D*) Strand transfer between circular ssM13 DNA and linear dsM13mp18 DNA. (*E*) Analysis of base pairing in heteroduplex DNA molecule consisting of M13mp18 and M13 DNA. The heteroduplex DNA molecule was generated by strand transfer between M13mp18 circular ssDNA and M13 linear dsDNA molecules. After strand transfer, a portion of the product was digested with restriction enzyme *Eco*105I. Lane 1, no digestion; lane 2, digestion by *Eco*105I.

37-nt deletion as well. The 37-nt deletion in circular ssDNA or linear dsDNA is 4050 bp away from the annealing region between these two DNA molecules. As shown in Fig. 4, circular dsDNA molecules, the products of the full strand transfer reaction, were found in the strand transfer reaction mediated by the gene *4* helicase regardless of whether the 37-bp deletion occurred in the dsDNA (Fig. 4*A*) or ssDNA (Fig. 4*B*) partner. However, the extent of strand transfer during the incubation period was consistently greater when the 37-bp deletion was located in the linear dsDNA partner.

To determine if a longer nonhomologous region has a more severe effect on strand transfer, joint molecules were constructed in which each of the DNA partners contained an 842-nt insert. In this instance the two DNA partners constituting the joint molecule were M13 DNA and M13mp18 DNA, the latter DNA containing an 842-nt insert not present in M13 DNA. The 842-nt insert is about 3.5 kb away from the annealing region. When the 842-nt insert was present in the circular single-stranded partner, gene *4* protein mediated strand transfer as well as when the two partners had identical sequences (Fig. 4*C*). In contrast, no strand transfer was observed when the insert was present in the linear duplex partner as measured by the absence of circular dsDNA (Fig. 4*D*).

Characteristics of Products of Strand Transfer. The above results show clearly that gene *4* protein-mediated strand transfer can proceed through long nonhomologous regions, provided that such regions are present in the ssDNA partner. We have also examined the products of strand transfer to determine if the proper base pairs have been formed downstream of the nonhomologous region. For this characterization we have used restriction enzyme analysis and direct visualization of the products by electron microscopy.

In the heteroduplex product molecule in which one strand is derived from M13 DNA and the other from M13mp18 DNA (containing an insert of 842 nt) there is a unique *Eco*105I site about 2000 bp downstream from the nonhomologous region. If base pairing is correct, the circular dsDNA, the product of strand transfer, should be cut by the restriction enzyme to yield linear DNA. As shown in Fig. 4*E* the product molecules were susceptible to cleavage with *Eco*105I as evidenced by the presence of linear dsDNA molecules that migrated slower than the linear dsM13 DNA as a result of the extra loop of 842 nt. The linear M13 dsDNA loses 921 bp after the *Eco*105I digestion and so it migrates faster than linear M13 dsDNA.

We have used electron microscopy to examine the structure of the product of strand transfer using a joint molecule consisting of circular single-stranded M13mp18 DNA and linear double-stranded M13 DNA. In addition to examining the product of full strand transfer at 20 min of incubation we have also examined an intermediate product of strand transfer found at 1 min of incubation. To facilitate the examination of the single-stranded region of the product we have incubated the DNA with *E. coli* SSB protein prior to electron microscopy. The intermediate found at 1 min of incubation (Fig. 5*A*) is a ∂-structure containing a partially duplex circle, a linear duplex segment, and a single-stranded arm arising from the junction of the single-stranded and double-stranded portion of the circle. This structure is consistent with that predicted from an earlier model for *E. coli* RecA protein-mediated strand exchange (34). The product of full strand exchange (Fig. 5*B*) consists of a circular duplex DNA molecule with a single-stranded loop where the size of the loop is compatible with that

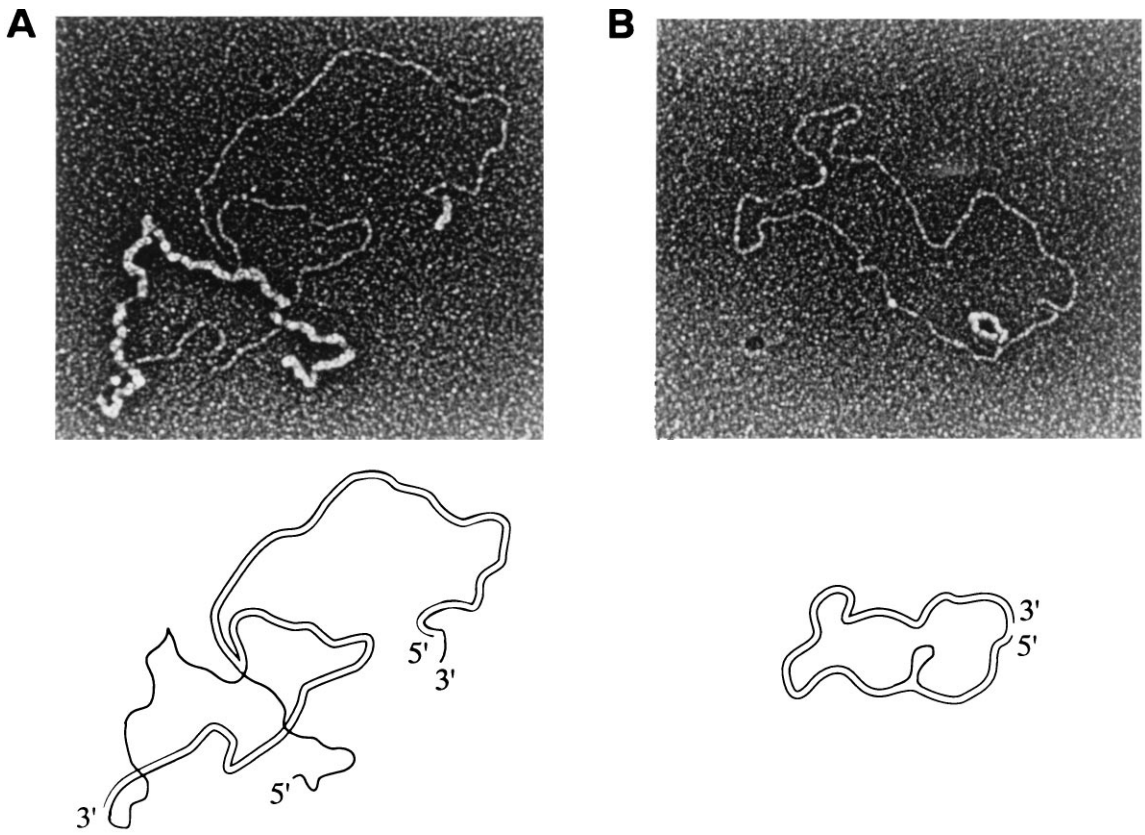

FIG. 5. Visualization of products of strand exchange. Joint molecules composed of M13mp18 circular ssDNA and M13 linear dsDNA were used as substrates for the strand transfer reaction. The strand transfer reaction mixtures were incubated at 32°C for 1 min or 20 min and then subjected to extraction with phenol to eliminate the gene *4* protein. DNA samples were incubated with *E. coli* SSB protein and analyzed by electron microscopy. (*A*) Intermediates of strand transfer found at 1 min of incubation. (*B*) Products of full strand transfer found at 20 min of incubation.

predicted above for the nonhomologous region found in M13mp18 DNA.

DISCUSSION

DNA helicases translocate unidirectionally on DNA and catalyze the unwinding of duplex DNA in a reaction fueled by the hydrolysis of a nucleoside triphosphate (35). The helicases were initially identified on the basis of their essential role in DNA replication. But in light of the number of cellular processes that require translocation and unwinding of DNA, their participation in other cellular processes such as priming of DNA synthesis, recombination, repair, and transcription should have been anticipated. For example, the ability of DNA helicases to unwind a segment of DNA is important in nucleotide excision repair of damaged DNA (36). In addition, the recent findings that the bacteriophage T4 and T7 helicases mediate strand exchange *in vitro* (1, 2) raises the possibility that this group of enzymes can mediate recombinational repair of damaged DNA that has escaped repair by photoreactivation or excision repair pathways. In this communication we show that the bacteriophage T7 gene *4* helicase can mediate the transfer of one strand of a duplex molecule to a homologous DNA strand when either molecule contains UV-induced pyrimidine dimers or insertions of nonhomologous DNA. The ability of the T7 helicase to efficiently use these DNA substrates in this recombination step is reminiscent of similar reactions mediated by the *E. coli* RecA protein (8, 9), a protein known to be involved in recombinational repair of DNA (32, 33).

In the experiment shown in Fig. 3, complete strand transfer occurred using joint molecules that had been subjected to UV irradiation which induced about 130–260 pyrimidine dimers per joint molecule. Since ssDNA and dsDNA are equally susceptible to formation of UV-induced pyrimidine dimers, each of the three strands in the joint molecule (Fig. 1) contains 43–86 lesions. In addition, examination of strand transfer at short times of incubation failed to show any decrease in the rate of strand transfer. This result contrasts with that observed with the RecA protein, where approximately 20 sec is required for the protein to bypass a pyrimidine dimer, a reduction in the rate of the RecA-mediated strand exchange of approximately 50-fold (9). T7 gene *4* protein clearly translocates along the linear strand that is not exchanged (37), and it probably also interacts with the linear strand to be exchanged. It seems likely that pyrimidine dimers do affect the rate of translocation and consequently strand transfer, since some bulky adducts are known to reduce or stop the movement of the gene *4* protein on ssDNA (38). Although it is possible that the sensitivity of our assay, the formation of products of complete strand transfer, is insufficient to detect a difference, a more likely explanation may lie in the nature of the DNA substrate. In the strand exchange reaction spontaneous branch migration could drive the strand transfer past the pyrimidine dimer even if the helicase is temporarily stalled at the lesion. In any case, considering the efficient bypass of pyrimidine dimers during T7 helicase-mediated strand transfer, DNA helicases in general may well play a major role in recombinational repair. In bacteriophage T4-infected cells, mutations in the viral gene *41*, the structural gene for the phage helicase, increase the sensitivity of the phage to UV irradiation (39).

The formation of heteroduplexes containing mismatched bases, insertions, and deletions must occur when such differences exist between the two DNA partners of strand exchange. We have shown that the T7 DNA helicase efficiently drives strand transfer through mismatched bases and relatively large regions of nonhomology. In the heteroduplex formed between

M13 DNA and fd DNA, 3% of the nucleotides are mismatched. Among these mismatches there are five sites at which two adjacent nucleotides are mismatched, one of three adjacent mismatches, and one region of 22 base pairs of which 8 are mismatches (27). No difference in strand transfer was observed when either DNA molecule was present as the circular ssDNA partner or as the linear dsDNA partner.

In the case of strand transfer between two molecules containing nonhomologous DNA regions the location of the nonhomologous region is extremely important. When the nonhomologous region, whether it is 37 or 842 nt long, is present in the single-stranded circular partner, full strand transfer proceeds as efficiently as when the nonhomologous region is absent. In the case of the 842-nt-long insert the nonhomologous region appears as a single-stranded loop in the product of complete strand transfer as visualized by electron microscopy. However, when the nonhomologous region is present in the linear dsDNA partner strand transfer is either slowed or incomplete, depending on whether the insert is 37 or 842 nt, respectively. When the insert is present in the duplex DNA molecule no strand transfer can occur until the helicase has progressed through the entire nonhomologous region and returned to a region of homology. One explanation for the cessation of strand transfer in this instance is that in the absence of homologous base pairing involving the partner molecules there is insufficient driving force for the helicase to continue. Alternatively, if the region of nonhomology is sufficiently long the nonhomologous region will simply reanneal as the helicase passes through.

The rate of recombination in cells infected with bacteriophage T7 is very high, reaching values of 20–40% between sites that are widely separated on the T7 genetic map (14). Such high levels of recombination appear to be catalyzed by phage-encoded proteins, since recombination efficiencies in phage-infected cells are not affected in the absence of *E. coli* RecA protein (17, 40). We have proposed, on the basis of *in vitro* studies, that the efficient recombination observed in phage-infected cells is mediated by the T7 gene *2.5* protein and the T7 gene *4* helicase (2). Furthermore, recombinational repair of dsDNA breaks in T7 phage-infected cells is very efficient (15, 16). The studies presented here show that the helicase-mediated strand transfer reaction has the capability to play a major role in postreplication recombinational repair. Definitive conclusions must await *in vivo* studies with gene *2.5* and gene *4* mutants.

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