A Single Nuclease Active Site of the *Escherichia coli* RecBCD Enzyme Catalyzes Single-stranded DNA Degradation in Both Directions*

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The RecBCD enzyme of *Escherichia coli* is an ATP-dependent DNA exonuclease and a helicase. Its exonuclease activity is subject to regulation by an octameric nucleotide sequence called χ. In this study, site-directed mutations were made in the carboxyl-terminal nuclelease domain of the RecB subunit, and their effects on RecBCD's enzymatic activities were investigated. Mutation of two amino acid residues, Asp1067 and Lys1082, abolished nuclelease activity on both single- and double-stranded DNA. Together with Asp1080, these residues compose a motif that is similar to one shown to form the active site of several restriction endonucleases. The nuclease reactions catalyzed by the RecBCD enzyme should therefore follow the same mechanism as these restriction endonucleases. Furthermore, the mutant enzymes were unable to produce χ-specific fragments that are thought to result from the 3′-5′ and 5′-3′ single-stranded exonuclease activities of the enzyme during its reaction with χ-containing double-stranded DNA. The results show that the nuclease active site in the RecB C-terminal 30-kDa domain is the universal nuclease active site of RecBCD that is responsible for DNA degradation in both directions during the reaction with double-stranded DNA. A novel explanation for the observed nuclease polarity switch and RecBCD-DNA interaction is offered.

The RecBCD enzyme of *Escherichia coli* (exonuclease V; reviewed in Refs. 1–3) is a trimeric, multifunctional enzyme composed of the RecB, RecC, and RecD subunits, whose calculated molecular masses are 134, 129, and 67 kDa, respectively (4–6). The enzyme is an ATP-stimulated ssDNA* endonuclease, an ATP-dependent ssDNA and dsDNA exonuclease, and a DNA helicase. RecBCD performs two important functions in the *E. coli* cell. Any linear dsDNA, such as the genomes of bacteriophages λ and T4, is quickly degraded by RecBCD upon entering the cell if it is not accompanied by a protective mechanism (7, 8). Furthermore, RecBCD helps *E. coli* to recover from double strand breaks in its chromosome during genomic replication (9). This function of the enzyme is believed to be important in maintaining the viability of *E. coli* (9). Strains deprived of RecBCD, such as V186 (ΔrecBCD) (10), are 10 times less viable than those possessing wild-type RecBCD (10, 11).

RecBCD faces a dilemma during its interaction with dsDNA: whether or not to degrade it completely. Invading foreign DNA should be completely destroyed, whereas the *E. coli* genome that has suffered a double strand break should be preserved via recombinational repair. Nature has an ingenious solution for the cell, in the form of an 8-nt sequence called χ (5′-GCTG-GTGG-3′ (12)). χ appears at high frequency in the *E. coli* chromosome, marking it for protection. The absence of χ in a dsDNA (e.g. in phage λ) defaults it to complete degradation. χ, originally discovered as a recombination hot spot (13, 14), stimulates recombination in its vicinity 5–10-fold (reviewed in Refs. 1 and 3). This stimulation is dependent on the RecBCD enzyme and has been found to arise from the ability of χ to suppress the nuclease activity of RecBCD and because of RecBCD’s ability to load RecA protein onto the newly unwound ssDNA (15, 16). Analyses on the *E. coli* chromosome have revealed that the density of χ is higher in the proximity of the origin of replication, oriC, and these χ sites are favorably oriented to protect oriC from degradative RecBCDs approaching from both sides (17).

The immediate effect of χ on RecBCD is mainly manifested in the dsDNA exonuclease activity (18). The dsDNA exonuclease function is a result of combined helicase unwinding and subsequent endonucleolytic cleavage on the unwound ssDNA (19). While unwinding, the enzyme preferentially cuts the strand that is 3′-ended at its entry site, nicking the 5′-ended strand only occasionally (18). The 3′-strand cleavage is greatly attenuated if the enzyme encounters χ from the 3′-side as the sequence is written above (20), but the helicase activity is left much unaltered. The ssDNA thus produced, especially the 3′-ended strand, becomes the substrate for RecA-mediated strand pairing and exchange (16, 18). In vitro, if the substrate DNA is 5′-radiolabeled, one can detect a χ-specific fragment that is the result of RecBCD’s last cut on the χ-containing, 3′-ended strand and subsequent unwinding of the dsDNA downstream of χ (18, 21). In addition, the enzyme can cleave the strand containing the complement of the χ sequence and 5′-terminated at the entry site, in a χ-specific manner (22), and cleavage of this strand was actually found to increase after RecBCD passes the χ sequence (23). These observations led to the proposal that χ, in addition to down-regulating RecBCD’s 3′-to-5′ ssDNA exonuclease activity, up-regulates its 5′-to-3′ ssDNA exonuclease activity on the other strand. Moreover, it was proposed that two nuclease active sites, responsible for degradation in each direction, might exist in RecBCD (23).

Recent work has shown that the RecB subunit, in addition to its DNA helicase activity (24), has a critical role in the intricate...
RecBCD nuclease reactions (25). RecB protein has been shown to be composed of two domains: a 100-kDa amino-terminal domain responsible for DNA unwinding and a carboxy-terminal 30-kDa domain responsible for DNA degradation (26). A mutation made to a conserved amino acid residue in the 30-kDa domain, Asp1080, completely abolished the nuclease activities of RecBCD, suggesting that there exists only a single nuclease active site in the RecBCD enzyme (25). In order to identify other essential amino acid residues involved in DNA hydrolysis and to verify the number of nuclease active sites in RecBCD, we have conducted site-directed mutagenesis studies on other conserved amino acid residues in the RecB C-terminal 30-kDa domain and examined the DNA cleavage properties of the mutants obtained. We isolated two mutants, RecB101672CD and RecB101825CD, which are inactive as nuclease but active as helicases. Neither mutant produces χ-specific fragments from either strand of a χ-containing dsDNA substrate. The results, consistent with the findings of Yu et al. (25), show that the nuclease active site on the RecB 30-kDa domain is the universal nuclease active site of RecBCD that is responsible for ssDNA degradation in both directions. We also offer a novel explanation for both the observed nuclease polarity switch and for RecBCD-χ interaction.

**EXPERIMENTAL PROCEDURES**

**Materials**

Restriction endonucleases, calf intestinal phosphatase, and T4 DNA ligase were obtained from Promega or New England Biolabs and were used as recommended by the suppliers. Single-stranded circular M13 DNA was a gift from Jehanne Souaya of this laboratory and was purified as described (27). Bacteriophage T4 with an amber mutation in gene 2 (T2, χ phage) was a gift from Dr. Gerald R. Smith at the Fred Hutchinson Cancer Research Center (Seattle, WA).

**Methods**

Site-directed Mutagenesis of the recB Gene—Plasmid pFS9kb (9475 bp) was used as the template for site-directed mutagenesis. It was constructed by deleting the 13,220-bp BglII fragment from the plasmid pFS11-04, which is pBR322 containing an 18.5-kilobase ptrIII-recB-recD-argA chromosomal fragment (28). The QuikChange site-directed mutagenesis kit (Stratagene) was used to create the mutations. The oligonucleotides used (obtained from Life Technologies, Inc.) were as follows: 5'-CAT ATG TTA AAA GCC TTT ATC GTC GTG TTT TTC CGC C-3' and 5'-CCG GAG CAG CAC AGC AAT GTA GCC TTA CAT G-3' for the D1067A mutation; 5'-GGT TAT TAC CTC GTG GAC TTT AAA TTC AAG TGG-3' and 5'-CTT CAA GCT GCA ATT GAC TGC GTA ATA ACG-3' for the Y1081A mutation; 5'-TG TAT TAC CTC GTC GAC TTA AAC ACG TGG-3' and 5'-CTT CAA GCT GCA ATT GAC TGC GTA ATA ACG-3' for the Y1081A mutation; 5'-TAC CTC CTA GCT CAA TAC TGG TGG-3' and 5'-CCG GAG CAG CAC AGC AAT GTA GCC TTA CAT G-3' for the Y1081A mutation; 5'-CTG CCG CAG CAC AGC AAT GTA GCC TTA CAT G-3' for the Y1081A mutation; 5'-CGT CCG CAG CAC AGC AAT GTA GCC TTA CAT G-3' and 5'-TAV TAA CAT G-3'.

The ssDNA and dsDNA exonuclease assays were conducted by measuring the production of acid-soluble DNA fragments as described (32). The dsDNA exonuclease assay used 0.2 nM RecBCD or 2 nM mutant enzyme. The substrate DNA was 5.06 μM [3H]PvSm19 (6250 bp (33)) linearized by BglII digestion. The ssDNA exonuclease assay used 1 nM RecBCD or 10 nM mutant enzyme. The substrate DNA was 5.06 μM [3H]PvSm19, linearized by BglII digestion and heat-denatured. The mutations were confirmed by DNA sequencing. The reaction products were performed, and the reaction products were analyzed on native agarose gels, as described (23), using 1.15 nm HindIII-linearized pBR322-χ DNA (12, 2 μM SSB protein, 0.115 nM RecBCD, or 0.8 nM mutant enzyme at varying magnesium acetate and ATP concentrations.

**RESULTS**

**Mutagenesis on the recB Nuclease Domain**—We first made four mutations, D1067A, Y1081F, K1082Q, and Y1114F, by site-directed mutagenesis in the carboxy-terminal 30-kDa nuclease domain of RecB (26) based on the sequence alignment shown in Fig. 1 and that published by Aravind et al. (34). The four mutations were then cloned into the plasmid pFS11-04 (28), which is pBR322 containing the recB, recC, and recD genes. Two of the mutations, D1067A and K1082Q, must be deleterious to E. coli, since we were unable to obtain strains of V186 (ArcBCD) containing either mutant pFS11-04 plasmid, even by using the high efficiency electroporation method. This negative effect on cell viability seems to be recessive to the wild-type recBCD allele, since DH5 cells (recBCD') containing the mutant pFS11-04 plasmids did not appear to have any growth defects. The expression of the recB, recC, and recD genes in pFS11-04 utilizes their natural promoters contained in the deletion (29) and is affected only by the copy number of pBR322 (25 copies/cell (29)). The four mutations were then transferred to a lower copy number plasmid, pABC, which is pACYC184 (10–12 copies/cell (29)) with the same insertion as pBR322. The four mutations were transferred to a lower copy number plasmid, pABC, which is pACYC184 (10–12 copies/cell (29)) with the same insertion as pBR322 (25 copies/cell (29)). The four mutations were then transferred to a lower copy number plasmid, pABC, which is pACYC184 (10–12 copies/cell (29)).
on the World Wide Web) to search the nonredundant protein data base subset of GenBankTM. The C-terminal regions of these proteins were then
aligned using the program CLUSTAL W (Ref. 60; available on the World Wide Web). Residues chosen for mutagenesis (Asp 1067, Tyr1081, Lys1082, Arg1069, 2
RecB, 2

alignment, and GenBankTM or Swiss-Prot accession numbers are as follows:

x

The data show that the RecB D1067ACD and

highly active. The data show that the RecB D1067ACD and

purified RecB D1067ACD and RecB K1082QCD mutants

under some reaction conditions using the 5’-labeled pBR322 F strand containing a single χ sequence (Fig. 5A): an ~1400-nt fragment extending from χ to the 5’-end (“downstream” fragment) and an upstream χ-specific fragment (>2900 nt) that results from cleavage of the χ-lacking strand near χ (lower strand in Fig. 5A; Ref. 23). The downstream χ fragment is produced because the 3’-5’ exonuclease of RecBCD is down-regulated after the enzyme encounters χ (18). The upstream fragment is proposed to result from activation by χ of a 5’-3’-specific exonuclease activity of RecBCD, since no discrete 3’-labeled fragment was detected from this strand (23). The degradation of both strands may indicate the existence of two distinct nuclease activity sites in RecBCD (23). We hypothesized that our RecB1067ACD and RecB1082QCD mutants would be disabled in producing the downstream χ fragment, as has been shown for the RecB1060ACD mutant (25), but that both should still be able to produce the upstream χ fragment if a separate 5’-3’ exonuclease activity exists in RecBCD. To test this hypothesis, we examined RecBCD-χ interactions under the same reaction conditions used by Anderson et al. (23).

As shown in Fig. 5, the wild-type RecBCD enzyme produces both the upstream and downstream χ bands as observed previously at the ATP and Mg2+ concentrations tested (23). However, the purified RecB1067ACD and RecB1082QCD mutants

Table I

<table>
<thead>
<tr>
<th>Strain</th>
<th>T2 phage titer*</th>
<th>Relative sensitivity</th>
<th>Nuclease activity†</th>
</tr>
</thead>
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<tr>
<td>V186 (Arc-BCD)</td>
<td>3 × 10^9</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>V186 (pa-BCD) (recBCD)</td>
<td>1.5 × 10^9</td>
<td>10^-6</td>
<td>3.3</td>
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<tr>
<td>V186 (pa-B1067ACD)</td>
<td>6 × 10^6</td>
<td>0.3</td>
<td>ND</td>
</tr>
<tr>
<td>V186 (pa-B1081QCD)</td>
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<td>&lt;5 × 10^-7</td>
<td>3</td>
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<tr>
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<td>&lt;10^3</td>
<td>&lt;5 × 10^-7</td>
<td>4</td>
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<tr>
<td>V186 (pa-B1082QCD)</td>
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<tr>
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<td>10^-6</td>
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</tr>
<tr>
<td>V186 (pa-B1082QCD)</td>
<td>&lt;10^3</td>
<td>&lt;5 × 10^-7</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* High phage titer indicates low nuclease activity in vivo. The results are averages of two separate experiments for all mutants except the Y1081A and Y1114A mutants, which were measured only once. Individual experiments agreed to within a factor of 2 or better.

† Nuclease activity measured in crude cell extracts using linear double-stranded [3H]DNA. The amount of acid soluble DNA produced with no ATP (~0.45–0.9 µm nt) was subtracted from that produced with 40 µm ATP to give RecBCD activity.

ND, none detected. Soluble DNA produced with ATP was equal to or less than that without ATP.
produced neither fragment under the wide range of Mg$^{2+}$ and ATP concentrations tested. This was not due to an inability of the mutants to bind or unwind their substrate DNA, since full-length ssDNA was clearly produced. The only reasonable explanation is that the ability of the mutant enzymes to degrade DNA strands of both polarities was abolished. This establishes that the single nuclease active site in the RecB 30-kDa domain is responsible for producing both $\chi$-specific bands and, hence, degradation of ssDNA of both polarities during the reaction with dsDNA.

**DISCUSSION**

*The Nuclease Active Site in RecBCD: Comparison with Other Nucleases—*Three of the amino acid residues of RecB altered in this study, Asp$^{1067}$, Lys$^{1082}$, and Tyr$^{1114}$, along with Asp$^{1080}$, previously changed to alanine by Yu et al. (25), are highly conserved among RecB homologs (Fig. 1) and a number of other proteins that have been called the “RecB nuclease domain” family (34). Tyr$^{1114}$ is conserved in all of the family members and was speculated to act as a nucleophile as seen in topoisomerases (34). The fifth residue, Tyr$^{1081}$, is an aromatic residue (Tyr or Trp) in the RecB homologs (Fig. 1) but is not conserved among the other members of the family (34). The results of changing these Tyr residues to either Phe or Ala show that neither has an essential role in the nuclease reaction catalyzed by the RecBCD enzyme.

The other three residues, Asp$^{1067}$, Lys$^{1082}$, and Asp$^{1080}$ (25), are essential for the nuclease activity of RecBCD. Mutation of each of these residues produces a holoenzyme that has a high level of DNA helicase activity but is completely disabled as a nuclease on all DNA substrates. Thus, the single nuclease active site in the RecB 30-kDa domain is responsible for producing both $\chi$-specific bands and, hence, degradation of ssDNA of both polarities during the reaction with dsDNA.

Interestingly, these three residues compose a motif that is...
quite similar to an active site motif (PDX_{6-30}(D/E)ZK, where X is any residue and Z is often a hydrophobic residue) that has been found in several restriction endonucleases, including EcoRI, EcoRV, PvuII, BglI, and FokI (35, 36); in the 5′-3′ exonuclease of bacteriophage λ (37); and in the E. coli MutH protein (38). The three-dimensional structure of each of these enzymes has been solved. Despite the overall lack of significant sequence similarity and the functional differences among them, they share a common core structure, and the structural arrangements of these residues in the active sites are strikingly similar (35–39). Replacement of these amino acid residues in the restriction enzymes rendered them inactive or nearly inactive, not in binding, but in cleaving their corresponding DNA substrates (36).

Given the fact that the RecB^{D1067A}, RecB^{D1080A}, and RecB^{E1082Q} mutants are inactive as nucleases, it is reasonable to deduce that these mutated residues fulfill similar functions in the nuclease active site of RecBCD as their counterparts do in the restriction enzymes and that the phosphodiester bond hydrolysis reaction catalyzed by RecBCD follows a mechanism similar to that of these restriction enzymes. The acidic residues are thought to coordinate the Mg^{2+} ion that is essential to phosphodiester bond hydrolysis (35, 36). It is likely that the Mg^{2+} ion polarizes the phosphate and enhances its susceptibility to nucleophilic attack. The lysine residue also lies close to the phosphate (35, 36) and perhaps stabilizes the pentacovalent phosphorus in the transition state by compensating its extra negative charge.

RecBCD-dsDNA Interaction—This work, as well as previous studies on the RecB protein structure and the RecBCD nuclease specificity, raises a number of important mechanistic questions about RecBCD. First is the question of how the very weak nuclease activity of the RecB subunit alone, and especially the nuclease domain itself (25, 40), is enhanced by RecC and especially by RecD. Second, how is a single nuclease active site able to cleave DNA with both 3′-5′ and 5′-3′ polarities? Finally, how does a simple 8-nt χ sequence regulate the enzymatic activities of RecBCD? We propose a model (Fig. 6) that addresses each of these questions.

The RecB subunit (Fig. 6A), although a weak helicase by itself (24), is the major subunit of RecBCD that is responsible for DNA unwinding (41). The N-terminal 100-kDa domain of RecB, bearing all of the helicase motifs (26, 42), is responsible for DNA binding and unwinding. The C-terminal 30-kDa nuclease domain of RecB has very weak nuclease activity (26, 40), in part due to low affinity for DNA (25, 26). However, the activity of the 30-kDa domain when attached to a DNA binding domain (either the 100-kDa RecB domain or the phage T4 gene 32 protein (25)) is still very low, indicating that there must be additional factors that contribute to its low activity.

RecBC is a much more processive helicase than RecB (43, 44) and is capable of unwinding DNA at rates very close to that of RecBCD (45). RecB's existing DNA binding sites may be optimized, and alternative sites may form, either on RecB or in the interface between RecB and RecC. RecB in RecBC is not susceptible to proteolytic degradation, suggesting that RecC "shields" the hinge region between the two domains of RecB and may make contacts to both domains. However, RecC does not greatly activate the nuclease active site on the 30-kDa domain, since RecBC is still a weak nuclease (26, 46, 47).

The addition of RecD to RecBC produces a potent dsDNA exonuclease (46, 48). RecBC can be readily reconstituted in vitro by mixing the two subunits (43), suggesting that there is no energetically difficult conformational change that must occur. In contrast, the reconstitution of RecBCD from RecBC and RecD is a much slower process (32). This suggests that the formation of the holoenzyme cannot be viewed as the simple docking of the last subunit, RecD, to a preformed RecBC complex. Considerable conformational rearrangements must occur in RecB and/or RecC to accommodate the RecD subunit, and it follows that the three-dimensional structures of RecB and/or

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Figs. 5. The RecB^{D1067A} and RecB^{E1082Q} enzymes unwind dsDNA but do not produce χ-specific fragments. A, HindIII-linearized pBR322 χ F substrate structure. A RecBCD enzyme that starts at the right-hand end of the pBR322 χ F molecule can recognize the χ sequence and produce two 5′-32P-labeled fragments: the downstream χ fragment from the χ-containing upper strand and the upstream χ fragment from cleavage of the lower strand to the 3′ side of the χ sequence. The distance from the χ site to the 5′-end on each strand is indicated. B, Reaction mixtures contained 0.115 nM RecBCD or 0.8 nM mutant enzymes, 1.15 nM (molecules) HindIII-linearized 5′-32P-labeled pBR322 χ F, 2 μM SSB, and the indicated ATP and magnesium acetate concentrations. Reactions were started by adding the wild type or mutant enzymes, incubated at 37 °C for 4 min, quenched, and loaded onto a 1% agarose gel. The gel was run at 1.4 V/cm for 15 h, dried, and analyzed using a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA). Markers loaded in the leftmost lane (M) were as follows: full-length dsDNA (single-stranded, heat-denatured HindIII-linearized 5′-32P-labeled pBR322 χ F DNA) and 5′-32P-labeled pBR322 χ F DNA digested with both HindIII and Aval and then heat-denatured. Aval cuts 75 bp away from the χ sequence, and the 5′-32P-labeled HindIII/Aval fragments are 1396 and 2965 nt.
RecC in RecBCD must not be the same as they are in the RecBC protein. We believe RecD makes important contacts with the 30-kDa domain that could fine tune its structure into a more active nuclease conformation (26), and/or RecD could provide a key residue to complete the active site that is made up largely of the residues that we have altered in RecB.

The question of how a single nuclease active site in RecB can degrade both unwound strands of a duplex, and therefore ssDNA with both 3′-5′ and 5′-3′ polarities, can be resolved if the newly unwound 5′-terminated strand forms a loop that enables it to sit with the same 3′-5′ orientation across the nuclease active site as does the 3′-strand (Fig. 6, B and C). The looped ssDNA can still channel through RecBCD as the enzyme unwinds the dsDNA, and looping can therefore make this DNA strand a perfect substrate for the single 3′-5′ exonuclease activity residing on the RecB 30-kDa domain. The RecD subunit was cross-linked to the 5′-terminated strand in static complexes (49), and thus we suggest that this strand may be bound by RecD and directed to the nuclease active site. A proposed movement of the 30-kDa domain about a flexible peptide linking it to the N-terminal domain (26) could also have a role in determining which strand of a duplex gains access to the nuclease site for cleavage. However, to the extent that such movement occurs, the putative protein-protein interactions that enhance the nuclease activity must remain intact, at least under the conditions where DNA cleavage continues after χ.

The RecBCD nuclease strand-switching that is effected by χ under some reaction conditions must then be explained in the context of a single nuclease active site. We propose that when the ssDNA binding site that is engaged in DNA unwinding sees χ, it holds onto χ so that the unwound ssDNA no longer runs through the enzyme to the nuclease active site but instead loops out between the helicase active site at the front of RecBCD and the ssDNA binding site occupied by χ (Fig. 6D). In this case, the nuclease domain cannot cut the 3′-ended strand, not because its nuclease activity is turned off but because it can no longer meet the strand. With the 3′-ended strand excluded, the 5′-ended strand can now gain more access to the nuclease active site, and consequently the 5′-3′ exonuclease activity is seemingly turned on. Rather than χ up-regulating the 5′-3′ and down-regulating the 3′-5′ exonuclease activity, we regard the nuclease polarity switch of RecBCD as a simpler process, the turning point of which is a high affinity DNA-protein interaction between χ and the ssDNA binding site of RecBCD.

We thus envision χ as an oligonucleotide that has higher binding affinity to RecBCD than does any other ssDNA sequence. Several observations are consistent with a sequence-specific interaction of RecBCD with χ. A mutation in the RecC subunit produces a RecBCD holoenzyme that is not affected by the canonical χ sequence but that instead responds to a different χ-like sequence, as might be indicative of a direct protein-DNA interaction (50, 51). Moreover, sequences have been identified in various bacteria that have the same effect as χ in E. coli and are specific for the RecBCD-like enzymes found in those bacteria (52–54), consistent with a protein-DNA interaction where the DNA sequence-specificity is determined by the binding site structure on the cognate protein. Finally, the effects of χ on RecBCD can persist beyond χ and can be more dramatic than the alteration in the nuclease activity described above. χ has been shown to reversibly inactivate RecBCD as both a helicase and a nuclease by an unknown mechanism under certain reaction conditions (55–57). This more profound effect on the enzyme suggests changes in protein conformation or subunit interactions that are most likely initiated by a specific χ-RecBCD binding interaction.

Finally, we note that a phenomenon similar to the strand switching that occurs with RecBCD has been seen with other enzyme-nucleic acid systems. Two examples pointed out recently are the proposed movement of the acceptor end of a tRNA between the aminoacylation site and the editing site on an aminocyl tRNA synthetase and the movement of a single-stranded DNA chain between the polymerase and proofreading sites on DNA polymerase I Klenow fragment (58). Both mechanisms rely on the flexibility of a single polynucleotide chain to allow its movement between two active sites on the enzyme. The difference is that in those instances a single bound polynucleotide strand moves between two different enzyme active sites, while we propose that two different single-stranded polynucleotide chains can move to one active site in RecBCD.

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