Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 6706–6711, June 1997
Biochemistry

The recombination hotspot Chi is recognized by the translocating RecBCD enzyme as the single strand of DNA containing the sequence 5′-GCTGGTGG-3′

PIERO R. BIANCO AND STEPHEN C. KOWALCZYKOWSKI*

Sections of Microbiology and of Molecular and Cellular Biology, University of California, Davis, CA 95616

Communicated by Peter H. von Hippel, University of Oregon, Eugene, OR, April 22, 1997 (received for review February 24, 1997)

ABSTRACT **The RecBCD enzyme of *Escherichia coli* functions in the seemingly disparate roles of homologous recombination and the degradation of DNA. Which of these two roles it assumes is regulated by the 8-base recombination hotspot, Chi. Using double-stranded DNA substrates that are heteroduplex at the Chi locus we have established the determinants for Chi recognition. Our results show that an actively translocating RecBCD enzyme requires only the sequence information in the 5′-GCTGGTGG-3′-containing strand to recognize and to be regulated by Chi. Furthermore, the RecBCD enzyme can translocate through DNA heteroduplex bubbles as large as 22 bases, and still recognize a Chi sequence embedded in this region. This implies that recognition of Chi occurs following the unwinding of the DNA.**

In *Escherichia coli*, the RecBCD enzyme (EC 3.1.11.5), also known as exonuclease V, plays several important and diverse roles in DNA metabolism (1–4). *In vitro*, the enzyme is both a destructive exo- and endo-nuclease, and a highly processive DNA helicase (1–6). The degradation of duplex DNA is coincident with the unwinding of double-stranded DNA (dsDNA) and is due to endonucleolytic cleavage of the unwound single-stranded DNA (ssDNA) (1, 7). Although both the nuclease and helicase activities are generally sequence independent, RecBCD enzyme is unusual in that it will also recognize Chi sites (Chi = crossover hotspot instigator) (8, 9), which are known hot spots for genetic recombination (10).

The Chi site (χ-site, or χ) is an 8-base element within dsDNA that stimulates RecBCD enzyme-dependent recombination in its vicinity (10–13). The sequence is either 5′-GCTGGTGG-3′, its complement, or both (14, 15). Stimulation of recombination occurs primarily to the 5′ side of the χ sequence as it is conventionally written, requires RecBCD enzyme, and, as such, plays a central role in the primary recombination pathway of *E. coli*. *In vitro*, the χ-site causes RecBCD enzyme to generate a defined DNA fragment, terminating 4–6 nt to the 3′ side of χ, on the 3′-terminated strand at the dsDNA break that is used as the entry point for the enzyme (8, 9). The generation of a χ-specific ssDNA fragment occurs due to continued unwinding of the dsDNA beyond χ and is orientation dependent; RecBCD enzyme must approach χ from the 3′-side as it is written (see Fig. 1*A*) for both recognition and specific fragment production to occur (16).

In addition to defining the locus for a defined DNA break, χ is a unique regulatory element that acts as an attenuator of the dsDNA exonuclease activity of the RecBCD enzyme (17–20). Upon interaction with χ during translocation, degradation of the strand containing the 5′-GCTGGTGG-3′ sequence is reduced at least 500-fold, while the helicase activity of RecBCD enzyme is unaffected (17, 19). The specific interaction between RecBCD enzyme and χ causes the translocating enzyme to pause at χ (19). This pausing is the result of a specific recognition event that alters the functional state of RecBCD enzyme and, simultaneously, ensures that DNA cleavage occurs at the χ-site. Continued unwinding by RecBCD enzyme generates a ssDNA molecule downstream from χ that is preferentially used in RecA protein-dependent homologous pairing reactions *in vitro* (17, 21). Thus the χ sequence is an unusual regulatory element that converts the RecBCD enzyme from a highly destructive nuclease-helicase to a recombinogenic helicase.

To recognize Chi, the RecBCD enzyme must identify this 8-nt element uniquely, while translocating at 1,000 bp/sec. Previous studies have used DNA substrates containing heteroduplexes, termed "bubble" duplexes, to study transcription initiation, elongation, and termination by *E. coli* RNA polymerase (22–29). In some of these studies (27–29) it was shown that RNA polymerase and antitermination Q of phage λ make base-specific interactions with primarily the single-stranded nontemplate strand of the initially duplex promoter. Here, we describe the use of two sets of bubble substrates, heteroduplex at the Chi-locus, designed to define two of the most fundamental aspects of the RecBCD–Chi recognition complex. First, since previous studies established that Chi is recognized only during the unwinding of dsDNA by RecBCD enzyme (8, 9), we asked which strand, or both, contain(s) the recognition elements? Second, we asked whether recognition occurs when the DNA is in the double- or single-stranded form—i.e., does the recognition of Chi occur before or after unwinding of the dsDNA? The results here show that the recognition determinants for the recombination hotspot Chi are contained within the ssDNA sequence 5′-GCTGGTGG-3′, and that this sequence element is recognized as a single-stranded entity—i.e., after DNA unwinding.

METHODS

Chemicals and Oligonucleotides. All buffers were made up in Nanopure water, 0.2-micron filtered, and autoclaved. Glycogen (used as a carrier in ethanol precipitations), ATP, and dNTPs were purchased from Boehringer Mannheim. AmpliWax PCR Gems were from Perkin–Elmer and [γ-^{32}P]ATP was from NEN/DuPont. *Eco*RI restriction enzyme, T4 polynucleotide kinase, Vent DNA polymerase, and the CircumVent Thermal Cycle DNA sequencing kit were from New England Biolabs. Oligonucleotides used in the polymerase chain reaction (PCR) were synthesized on a Milligen/Biopore DNA synthesizer (Millipore) and gel purified using denaturing polyacrylamide gels. Following purification, oligonucleotide primers were stored in TE buffer (10 mM Tris·HCl/1 mM EDTA

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; Chi, crossover hotspot instigator; SSB, single-stranded DNA binding protein.

*To whom reprint requests should be addressed. e-mail: sckowalczykowski@ucdavis.edu.

buffer, pH 8.0), at −20°C. Concentration of primers was determined using an A_{260} of 1 = 33 μg/ml.

Proteins. RecBCD enzyme was purified using a published protocol (5), except that a Mono-Q column was used as the final purification step. The specific activity of the RecBCD enzyme used in these assays was 1.01×10^5 units/mg, and the preparation was 100% active, as determined using a spectrofluorometric helicase assay (5). RecA protein and the single-stranded DNA binding protein (SSB) were purified using standard laboratory procedures (30). Protein concentration was determined using ε = 40,000 $M^{-1}\cdot cm^{-1}$ for RecBCD, ε = 27,000 $M^{-1}\cdot cm^{-1}$ for RecA protein, and ε = 30,000 for $M^{-1}\cdot cm^{-1}$ for SSB.

Duplex DNA. The plasmids pBR322 and pBR322-χ^+E were purified using alkaline lysis (31) followed by isopycnic centrifugation in cesium chloride gradients (32). The plasmids pBR322 (χ°) and pBR322-χ^+E differ by only a single base at the χ-site: 5′-GCTGGATGG-3′ for χ° and 5′-GCTGGTGG-3′ for χ^+E (33). These plasmids were linearized with *Eco*RI and used as templates in PCR without further purification. The oligonucleotides used in these reactions annealed at position 882 for the left primer and at 1,294 for the right primer (numbering from the standard pBR322 sequence), generating a 413-bp fragment, with the χ-site asymmetrically placed 110 bp from one end (see Figs. 1*A* and 2*A*). The products from these reactions were separated by electrophoresis in 1% agarose gels, isolated via electroelution, and reamplified using the same left and right primers to produce a stock of 413 bp χ° or χ^+E DNA that was used to generate substrates in subsequent PCR reactions. All PCRs used Vent DNA polymerase to minimize PCR-generated mutations and also to ensure that substrates possessed blunt ends. Reactions were conducted using a hot-start PCR protocol with AmpliWax beads in a DNA Thermal Cycler (Perkin–Elmer/Cetus).

M-8 and M-22 Duplex Substrate Construction. The strand overlap extension reaction (34) was used to generate two variants of χ^+E DNA. The first reaction mutated the 8 bases corresponding to the χ-site, so that each purine or pyrimidine was converted to the other purine or pyrimidine. This reaction produced a 413-bp substrate designated M-8 (see Fig. 1*B*). The second reaction mutated the 8 bases corresponding to the χ-site and 7 bases on each side, generating a 22-base alteration to produce a 413-bp substrate designated M-22 (Fig. 1*B*). To minimize any potential contamination from residual χ-containing DNA, the substrate used to generate the M-8 and M-22 substrates was the 413-bp χ° PCR product. Following the final strand overlap extension reaction, the M-8 and M-22 substrates were subjected to electrophoresis in 1% agarose gels, electroeluted, and reamplified to generate a substrate stock using the same left and right primers as for the original 413-bp χ° or χ^+E DNA substrates. The mutations in the M-8 and M-22 substrates were confirmed by DNA sequencing using the Circum-Vent Thermal Cycle DNA sequencing kit.

Homo- and Heteroduplex DNA Substrate Construction. Duplex DNA 413 bp in length that was either χ^+, χ°, M-8, or M-22 (Fig. 1*A*) was used as a template in single-stranded PCR using individual 5′-end labeled primers. Following PCR, each single-stranded product was phenol extracted, ethanol precipitated, resuspended in TE, and subjected to electrophoresis in denaturing polyacrylamide gels. Each 413-nt length fragment was identified, excised from the gel, and isolated by electroelution. The appropriate strands were mixed in stoichiometric amounts and annealed using RecA protein and ATP, using conditions as described by Menge and Bryant (35). Buffers were then exchanged using S-200 microspin columns (Pharmacia). Duplex DNA formation was then determined by nondenaturing PAGE. Under standard conditions, 80–100% of the input ssDNA was converted into duplex DNA (data not shown).

Chi-Fragment Production Assay. If χ is present in the substrate and is recognized by RecBCD enzyme, then two discrete-sized products are produced: (*i*) full-length ssDNA originating from the 5′-terminal strand at the entry site, and (*ii*) a χ-specific fragment originating from the opposite strand, downstream of 5′-GCTGGTGG-3′ (19) (see Fig. 2). For the substrates used here, the χ-specific fragment is 110 nt in length, and full-length ssDNA is 413 nt. If the substrate is χ°, or if χ is approached from the incorrect direction, then only full-length ssDNA is produced (because the 3′-terminal strand is degraded and the 5′-terminal strand remains as full-length ssDNA) (19). The full-length DNA and χ-specific fragments are easily distinguished using denaturing polyacrylamide gels as described below.

Reactions (20 μl) contained 25 mM Tris-acetate (pH 7.5), 1 mM DTT, 2 mM magnesium acetate, 2 mM ATP, 2 μM SSB, 9 nM dsDNA ends, and 0.26 nM active RecBCD enzyme. Incubations were conducted at 25°C for the indicated times and stopped by the addition of a mixture of phenol/chloroform/isoamyl alcohol (25:24:1). Stopped reactions were vortex mixed and centrifuged, and the aqueous phase was transferred to a new tube and mixed with an equal volume of formamide loading dye. The mix was heated to 100°C for 5 min and subjected to electrophoresis in 6% denaturing polyacrylamide gels. Following electrophoresis, gels were dried, exposed to PhosphorImager screens, and analyzed using a Molecular Dynamics Storm 840 PhosphorImager with IMAGE QUANT software. The migration of full-length ssDNA and χ-specific fragments in gels was determined relative to the position of molecular weight markers (data not shown). The production of χ-specific fragment was calculated as a percent of the total input dsDNA.

RESULTS

Which DNA Strand Defines χ? We first wanted to determine which strand defines χ; in other words, is it the strand 5′-GCTGGTGG-3′, its complement 3′-CGACCACC-5′, or are both strands required for recognition and χ-dependent fragment production? To answer this question, 413-bp homoduplex substrates that either contained χ (i.e., χ^+) or were devoid of χ (i.e., χ°), were used as templates in separate, single-stranded PCR reactions, with 5′-end labeled primers to amplify either the 3′-terminated strand relative to the entry point of RecBCD enzyme (henceforth known as the top strand), or the 5′-terminated strand (henceforth, the bottom strand) of each 413-bp fragment (Fig. 1). Following this, the top and bottom strands of the four reactions were annealed using the RecA protein of *E. coli* to yield the four possible dsDNA combinations shown in Fig. 1 (homoduplex substrates A1 and A2; heteroduplex substrates B1 and B2). Each of these annealed substrates was then tested for χ recognition using χ fragment production assays. The results are shown in Fig. 2. As expected, a χ-dependent fragment was observed for the positive control, χ^+-top/χ^+-bottom (Fig. 2*B*, lanes 8 and 9), and no fragment was observed for the negative control, χ°-top/χ°-bottom (lanes 2 and 3). For the heteroduplex substrates, a χ-dependent fragment was produced in the reaction in which the top strand of the heteroduplex substrate contained the 8-base χ sequence, as seen in lanes 11 and 12 of Fig. 2*B*. No χ-dependent fragment was observed when the top strand was χ° and the bottom strand contained the χ-complement sequence (lanes 5 and 6). Similarly, no χ-specific fragment was detected when any of the individual single strands of DNA were tested (data not shown), confirming previous studies (8) that χ is recognized only by a RecBCD molecule that is actively translocating through dsDNA. Thus, recognition of χ resulting in the production of a χ-specific fragment by an actively translocating RecBCD enzyme requires only the sequence information present in the 3′-terminated, or top DNA strand.

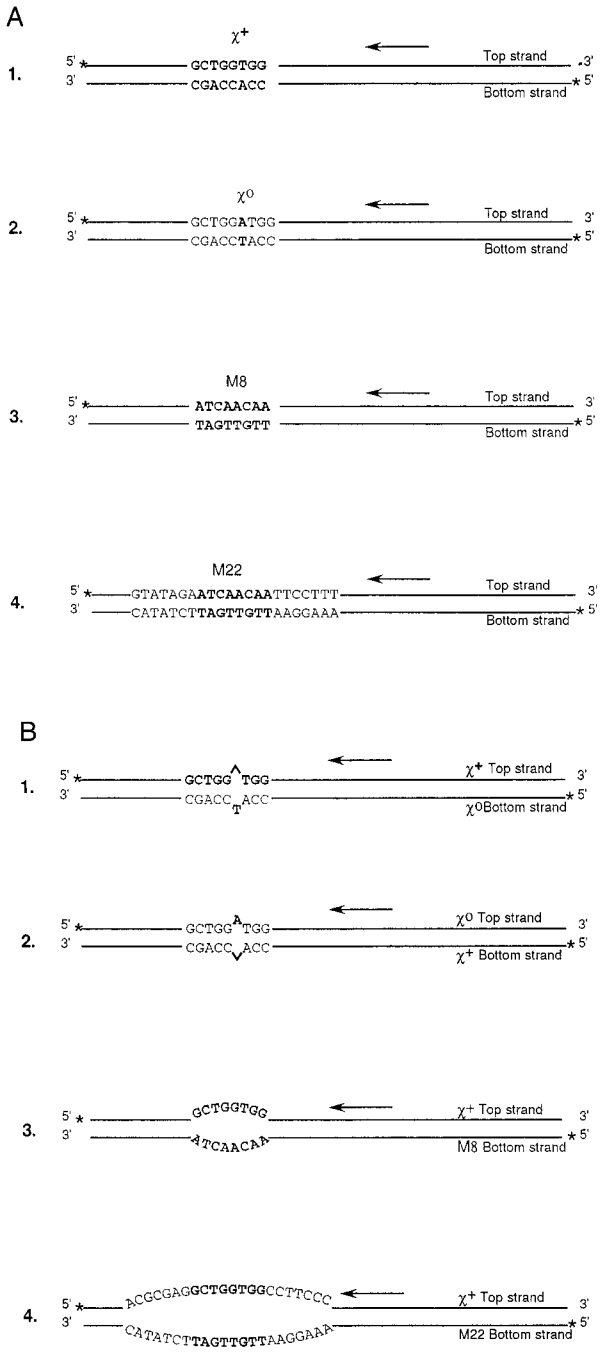

FIG. 1. Substrates used for χ-fragment production assays. (*A*) Homoduplexes. (*B*) Heteroduplexes. The χ^+/χ° and χ°/χ^+ DNA heteroduplexes contain a single base mismatch; χ^+/M-8 and χ^+/M-22, contain 8-and 22-base mismatches, respectively. The arrow above each substrate indicates the direction from which RecBCD enzyme must translocate for χ recognition to occur. Sequences in bold highlight both the χ^+ sequence and the positions where relevant differences with respect to χ^+ occur. For M-22, only 8 bases out of 22 of the mismatch, corresponding to χ-complement are highlighted. *, ^{32}P.

Is χ Recognized Within ssDNA or dsDNA? The above experiment defines which strand is being recognized by RecBCD enzyme; however, it does not determine whether the strand is recognized in the double-stranded (i.e., before the DNA is unwound) or single-stranded form (i.e., after the DNA is unwound). To determine the "strandedness" of recognition, DNA heteroduplex substrates containing 8- or 22-bp bubbles were created. Using strand-overlap-extension PCR (34), either the 8-base χ-site was mutated to produce M-8, or a 22-base region which includes the χ-site and the 7 bases on either side of χ was mutated to produce M-22 (Fig. 1*A*, substrates 3 and 4). A bubble size of 22 bases was selected because this is 1 base larger than the size of the footprint for RecBCD enzyme as determined by DNase I mapping (36); if the enzyme's footprint is not significantly altered during unwinding then, when it encounters a 22-base bubble, it should be entirely within a single-stranded region.

When ssDNA from substrate M-8 was annealed to ssDNA which did not contain the 8-base modification, an 8-base bubble was formed (Fig. 1*B*, substrate 3). Similarly, when substrate M-22 was used, and ssDNA from this fragment annealed to ssDNA which did not contain the mutation, a 22-base bubble was created with the χ sequence located in the center of the bubble (Fig. 1*B*, substrate 4). The positive control was a duplex fragment containing the χ sequence and its complement, while negative controls were the corresponding M8/M8 and M22/M22 homoduplexes. If RecBCD enzyme can translocate through these ssDNA bubble regions and if χ is recognized, then a χ-specific fragment will be produced, showing that recognition can occur when the DNA strands are single stranded; if no χ-specific fragments are produced, then recognition most likely occurs when the χ sequence is in the double-stranded form.

As expected, a χ-specific fragment was produced in the positive homoduplex control (Fig. 3*B*, lane 2) and no χ-specific fragment was produced in either of the negative controls (lanes 8 and 10). A χ-specific fragment was produced in the heteroduplex bubble reactions only when the χ sequence was present in the top strand (lanes 4 and 6). In addition, the yield of χ-specific fragments was similar for both the heteroduplex and homoduplex DNA substrates (6 ± 0.5% for $\chi^+/$M-8; 9 ± 2% for $\chi^+/$M-22; 5 ± 1% for χ^+/χ^+). No χ-specific fragments were produced when the χ sequence was absent (negative controls), or when the complement of χ was present in the lower strand (lanes 12 and 14). Since χ-dependent fragment production occurred in the χ-containing bubble substrates, and no other bubble substrates, this indicates that RecBCD enzyme does not simply translocate and degrade up to the bubble, and then stop; but rather it is able to translocate through the single-stranded region beyond χ and, more importantly, is able to recognize χ. Consequently, the recognition of χ by an actively translocating RecBCD enzyme requires sequence information in the 5′-GCTGGTGG-3′-containing strand, and these data strongly suggest that this determinant is recognized as a single-stranded entity after DNA unwinding.

DISCUSSION

In this paper, we make the following observations. (*i*) The RecBCD enzyme can translocate through DNA heteroduplexes up to 22 bp in size. (*ii*) Chi is recognized by a translocating RecBCD enzyme when this DNA sequence is in a single-stranded form, implying that recognition occurs after the DNA is unwound. (*iii*) These findings allow us to define the recombination hotspot Chi as the ssDNA sequence 5′-GCTGGTGG-3′.

In vivo, the recombination hotspot Chi was shown to be a cis-acting element that enhanced recombination promoted by the RecBCD enzyme (10–13). This element was later shown to be the duplex DNA sequence 5′-GCTGGTGG-3′ (14, 15). To more precisely define the molecular form of Chi, we utilized DNA substrates heteroduplex at the χ locus. These substrates allowed us to determine that, although Chi must be contained and recognized within dsDNA, it is simply the single-stranded sequence 5′-GCTGGTGG-3′ that constitutes the χ element. When χ is recognized by an actively translocating RecBCD enzyme approaching from the correct orientation, the predominant product is a downstream "top-strand" χ-specific fragment (19, 37). Formation of this fragment requires two

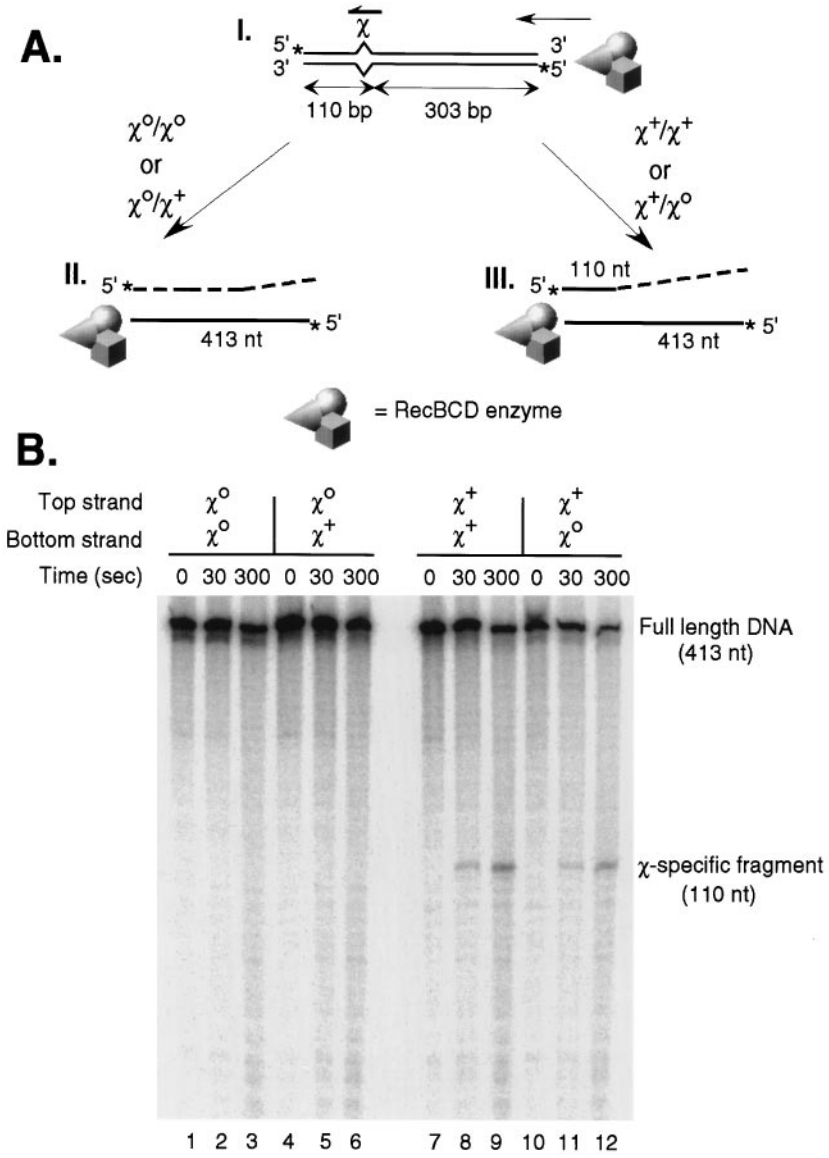

FIG. 2. Recognition of χ by RecBCD enzyme requires sequence information in the top strand. The substrates and expected products are shown in *A*; the results of the χ-specific fragment production assays using these substrates are shown in *B*. χ fragment production assays were performed as described.

sequential events: (*i*) the translocating enzyme must recognize Chi, and (*ii*) it must respond to this sequence by attenuating its nuclease activity while retaining its helicase activity. Our data show that the sequence information contained within the top strand was sufficient for both recognition and attenuation. Thus the single strand of DNA that contains the sequence, 5′-GCTGGTGG-3′, is the strand that defines the recombination hotspot Chi. Furthermore, this same strand is responsible for attenuating the 3′ → 5′ nuclease activity of RecBCD enzyme, and is also the strand which is preferentially used by RecA protein in strand exchange reactions *in vitro* (38).

Our results also define when, during the course of translocation, Chi is recognized. Recognition of the single strand of DNA containing Chi can occur either when the DNA duplex is still intact or when the DNA duplex has been unwound. If recognition occurs when the duplex is still intact, RecBCD enzyme must possess a sensing domain or subunit that precedes the helicase subunit of the enzyme and that reads the sequence information contained within one strand of the duplex. If recognition occurs following unwinding (in other words, when the DNA is in a single-stranded form), then a direct specific interaction between one of the subunits and the unwound, ssDNA containing χ, can occur. In either case, once this interaction has occurred, the recognition event is then transmitted to the enzyme to elicit the appropriate response. Our data from the 8- and 22-base bubble substrates, in which the χ-site is already single stranded before the enzyme encounters χ, strongly suggest that recognition of Chi occurs when the DNA is unwound.

Finally, our results demonstrate that RecBCD enzyme is fully capable of translocating through the DNA heteroduplex bubbles used here. If it were unable to do so, this would be observed as the production of apparent Chi-specific fragments in the χ° control reactions. This follows because the RecBCD enzyme would translocate up to the bubble region and then dissociate due to an inability to traverse such a discontinuity. A second enzyme molecule could then unwind from the opposite end of the molecule to produce what would appear to be a χ-specific fragment. This artifactual χ-specific fragment would be a consequence of the DNA heteroduplex bubble only, and production of this fragment would be independent of the presence of a χ-site within that bubble region; in other words, a "χ-specific fragment" would be produced in both the χ^+ as well as in the χ° substrates. This is not observed. Instead,

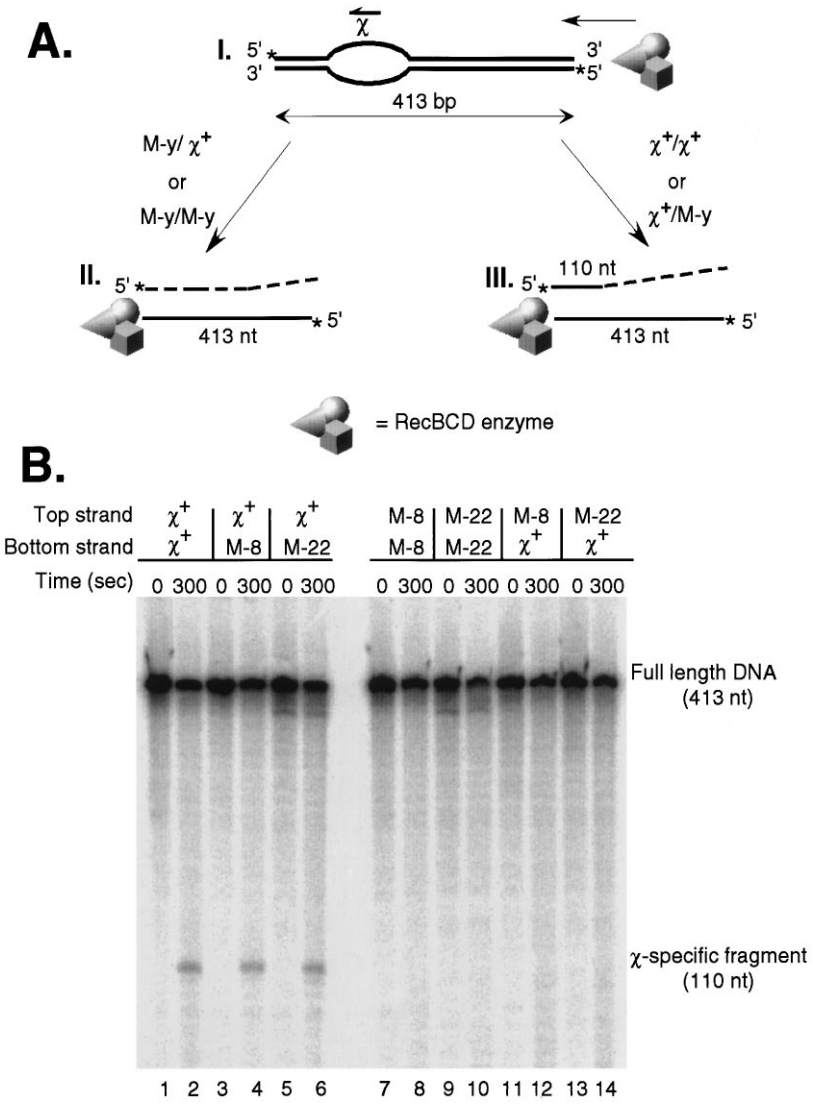

FIG. 3. The sequence determinant, 5′-GCTGGTGG-3′, can be recognized by a translocating RecBCD enzyme as ssDNA. The substrates and expected products are shown in *A*; the gel showing χ-specific fragment production is shown in *B*. Assays were conducted as for Fig. 2. M-y, M-8 or M-22.

our data demonstrate that only those substrates containing the single-stranded Chi sequence, 5′-GCTGGTGG-3′, in the top, or 3′-terminated strand of the bubble region, produced a χ-specific fragment. This means that RecBCD enzyme is both able to translocate through heteroduplex bubbles 1, 8, and 22 bp in size and, more importantly, is able to recognize and respond to a Chi sequence embedded within that bubble region.

We therefore propose the following model for χ recognition that is supported by our results using substrates which are heteroduplex at the Chi locus. The helicase subunit of an actively translocating RecBCD enzyme unwinds the duplex DNA in a nonspecific manner; this DNA, once unwound, is then fed to both the nuclease and Chi-recognition subunits (which may be the same subunit). The enzyme recognizes Chi as a single-stranded entity, responds to the sequence, and thereby converts RecBCD enzyme from a destructive nuclease–helicase to a recombinogenic helicase. This same strand, the 5′-GCTGGTGG-3′-containing strand, is then bound preferentially by RecA protein and used to promote DNA strand invasion of a homologous recipient.

Although the unique recognition of one strand of a DNA duplex is relatively unusual, it is not without precedent. A related behavior was reported for *E. coli* RNA polymerase, where the nontranscribed strand displays a regulatory function in both transcription initiation (29) and elongation (27). During promoter recognition, base-specific interactions between the σ^{70} subunit of RNA polymerase holoenzyme and the nontemplate strand are necessary for both tight binding and efficient functioning of the −10 region of the promoter (29). Ring *et al.* (28) showed that a translocating RNA polymerase holoenzyme could be induced to pause at a promoter-proximal sequence. Interestingly, base-specific recognition of Chi by the translocating RecBCD enzyme also results in a pause (19). In both situations, the paused enzyme undergoes a change, and, afterwards, continues translocation in a modified form.

These findings demonstrate that translocating enzymes that act on dsDNA such as RecBCD enzyme and RNA polymerase have the capacity to extract highly specific sequence information from a single strand of DNA, and thereby impart an asymmetry to a biological process by a novel recognition mechanism.

We thank Dan Anderson, Joel Brockman, Frederic Chedin, Jason Churchill, Deana Haddox, Frank Harmon, Alexander Mazin, Jim New, Bill Rehrauer, Erica Seitz, Tomohiko Sugiyama, Bob Tracy, Eugene Zaitsev, Elena Zaitseva, Ronald Baskin, Jon Scholey, and Mitchell Singer for their comments on the manuscript. This work was supported by National Institutes of Health Grant GM 41347 to S.C.K.

1. Telander-Muskavitch, K. M. & Linn, S. (1981) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), pp. 233–250.
2. Taylor, A. F. (1988) in *Genetic Recombination*, eds. Kucherlapati, R. & Smith, G. R. (Am. Soc. for Microbiol., Washington, DC), pp. 231–263.
3. Smith, G. R. (1990) in *Nucleic Acids and Molecular Biology*, eds. Eckstein, F. & Lilley, D. M. J. (Springer, Berlin), Vol. 4, pp. 78–98.
4. Kowalczykowski, S. C., Dixon, D. A., Eggleston, A. K., Lauder, S. D. & Rehrauer, W. M. (1994) *Microbiol. Rev.* **58,** 401–465.
5. Roman, L. J. & Kowalczykowski, S. C. (1989) *Biochemistry* **28,** 2863–2873.
6. Roman, L. J., Eggleston, A. K. & Kowalczykowski, S. C. (1992) *J. Biol. Chem.* **267,** 4207–4214.
7. MacKay, V. & Linn, S. (1976) *J. Biol. Chem.* **251,** 3716–3719.
8. Ponticelli, A. S., Schultz, D. W., Taylor, A. F. & Smith, G. R. (1985) *Cell* **41,** 145–151.
9. Taylor, A. F., Schultz, D. W., Ponticelli, A. S. & Smith, G. R. (1985) *Cell* **41,** 153–163.
10. Lam, S. T., Stahl, M. M., McMilin, K. D. & Stahl, F. W. (1974) *Genetics* **77,** 425–433.
11. McMilin, K. D., Stahl, M. M. & Stahl, F. W. (1974) *Genetics* **77,** 409–423.
12. Stahl, F. W., Crasemann, J. M. & Stahl, M. M. (1975) *J. Mol. Biol.* **94,** 203–212.
13. Stahl, F. W. & Stahl, M. M. (1977) *Genetics* **86,** 715–725.
14. Smith, G. R., Kunes, S. M., Schultz, D. W., Taylor, A. & Triman, K. L. (1981) *Cell* **24,** 429–436.
15. Smith, G. R., Comb, M., Schultz, D. W., Daniels, D. L. & Blattner, F. R. (1981) *J. Virol.* **37,** 336–342.
16. Taylor, A. F. & Smith, G. R. (1985) *J. Mol. Biol.* **185,** 431–443.
17. Dixon, D. A. & Kowalczykowski, S. C. (1991) *Cell* **66,** 361–371.
18. Dabert, P., Ehrlich, S. D. & Gruss, A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 12073–12077.
19. Dixon, D. A. & Kowalczykowski, S. C. (1993) *Cell* **73,** 87–96.
20. Kowalczykowski, S. C. (1994) *Experientia* **50,** 204–215.
21. Roman, L. J., Dixon, D. A. & Kowalczykowski, S. C. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3367–3371.
22. Daube, S. S. & von Hippel, P. H. (1992) *Science* **258,** 1320–1324.
23. Aiyar, S. E., Juang, Y. L., Helmann, J. D. & deHaseth, P. L. (1994) *Biochemistry* **33,** 11501–11506.
24. Aiyar, S. E., Helmann, J. D. & deHaseth, P. L. (1994) *J. Biol. Chem.* **269,** 13179–13184.
25. Daube, S. S., Hart, C. R. & von Hippel, P. H. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 9539–9543.
26. Daube, S. S. & von Hippel, P. H. (1994) *Biochemistry* **33,** 340–347.
27. Ring, B. Z. & Roberts, J. W. (1994) *Cell* **78,** 317–324.
28. Ring, B. Z., Yarnell, W. S. & Roberts, J. W. (1996) *Cell* **86,** 485–493.
29. Roberts, C. W. & Roberts, J. W. (1996) *Cell* **86,** 495–501.
30. Roman, L. J. & Kowalczykowski, S. C. (1989) *J. Biol. Chem.* **264,** 18340–18348.
31. Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7,** 1513–1523.
32. Vinograd, J. & Leibowitz, J. (1966) *J. Gen. Physiol.* **49,** 103–125.
33. Smith, G. R. (1983) *Cell* **34,** 709–710.
34. Horton, R. M. & Pease, L. R. (1991) in *Directed Mutagenesis: A Practical Approach*, ed. McPherson, M. J. (IRL, Oxford, U.K.), pp. 217–246.
35. Menge, K. L. & Bryant, F. R. (1992) *Biochemistry* **31,** 5158–5165.
36. Ganesan, S. & Smith, G. R. (1993) *J. Mol. Biol.* **229,** 67–78.
37. Dixon, D. A. & Kowalczykowski, S. C. (1991) *Cell* **66,** 361–371.
38. Dixon, D. A. & Kowalczykowski, S. C. (1995) *J. Biol. Chem.* **270,** 16360–16370.