Purification of a DNA Replication Terminus (ter) Site-binding Protein in Escherichia coli and Identification of the Structural Gene*

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In Escherichia coli cells, there is a protein that specifically binds to DNA replication terminus (ter) sites on the host and plasmid genome and then blocks progress of the DNA replication fork. We reported that extract of the cells carrying the plasmid with the tau gene, which was identified to be an essential gene for the termination reaction at the ter site, contained about an 8-fold increase in ter-binding activity of the plasmid-free cells. With improvement of the promoter region of the tau gene on the plasmid by site-directed mutagenesis, the host cells produced the ter-binding protein (Ter protein) over 2000-fold. Using these over-producing cells as the enzyme source, the Ter protein was purified to apparent homogeneity. Molecular mass 36,000, amino-terminal amino acid sequence (45 residues) and composition of the protein were in good agreement with those deduced from DNA sequence of the tau gene. Footprinting using the purified Ter protein revealed a specific binding to the ter sequence.

A DNA replication terminus (ter) site on replicons is the position at which progress of the DNA replication fork is either arrested or is severely impeded. The ter sites required for termination of DNA replication are present on the plasmid R6K genome and also on the bacterial chromosome of Escherichia coli and Bacillus subtilis (Lovett et al., 1975; Crooa et al., 1976; Weiss and Wake, 1983; Iismaa et al., 1984; Monteiro et al., 1984; Kuempel et al., 1977; Louarn et al., 1979). Similar sites have been found in yeast and plant cells (Brewer and Alani, 1976; Weiss and Wake, 1983; Iismaa et al., 1984; Monteiro et al., 1984; Kuempel et al., 1977; Louarn et al., 1979). Thus, the ter site may play an important physiological role(s).

To block the progress of the DNA replication fork, two factors are required, one of which is the ter sequence on the DNA molecule. Properties of the ter site present in the E. coli system are as follows. (i) All ter sequences are essentially the same, and their consensus 22-bp sequence is 5'-(A/T)(G/C/T)G/TAGTTACAACTPy(A/T)/C(A/T)(A/T)(A/T)(A/T)(A/T)-3'. The sequence was represented by (G/C) (Horiuchi and Hidaka, 1988; Hidaka et al., 1988; Hill et al., 1988b). (ii) The sequence has activity that inhibits travel of the replication fork in a specific direction (Horiuchi and Hill, 1988; Hidaka et al., 1988; Hill et al., 1988b). In the above orientation, only the fork, traveling from right but not left, is inhibited. (iii) A pair of the two ter sites is arranged in an inverted position. In the R6K plasmid, a pair of two terR sites (terR1 and terR2) is arranged in an inverted position (5'--3') 73 bp apart (Horiuchi and Hidaka, 1988; Hill et al., 1988b); in the E. coli chromosome, four terC sites (terC1,2,3, and 4) are located at the opposite region of the unique replication origin (oriC) and are arranged as (5'--3') 275 kilobases apart between the nearest pair of the terC sites (Hidaka et al., 1988). (iv) When the terC site is cloned into the ColEl derivative vector in the orientation in which the unidirectional replication fork starting from vector's origin is blocked at the terC site, presence of the site reduces the copy number of the hybrid plasmid because the site prevents the plasmid from completing DNA replication (Hidaka et al., 1988).

The ter-binding protein (Ter protein) is another factor essential for termination reaction. The ter-binding activity of the protein is controlled by a gene that we named tau (Kobayashi et al., 1989) or tus by Hill et al. (1988a, 1989); in cells carrying the plasmid on which the tau gene was located, the ter-binding activity was enhanced about 8-fold compared with the plasmid-free cells. On the other hand, in the tus-defective cells, which showed the termination-less phenotype, no ter-binding activity was evident, thereby suggesting that tau might be the structural gene for the Ter protein.

Hill et al. (1989) determined the nucleotide sequence of the tus gene, the expected molecular mass of the product of which was about 36 kDa. Complementarily, Sista et al. (1989) purified ter-binding protein, the molecular mass of which was somewhat less than 40 kDa. From footprinting experiments, they determined the sequence of the ter site covered by the protein. Although these data suggested that tau (tus) might be the structural gene for the Ter protein, conclusive evidence was not obtained.

We report here the Ter protein-overproducing system, purification of the Ter protein, and identification of the structural gene (tau) for the protein. We confirmed in DNase I footprinting experiments that the Ter protein does specifically bind to the ter sequence.

MATERIALS AND METHODS

Procedures—Restriction endonucleases, T4 ligase, T4 polynucleotide kinase, and DNA polymerase large fragment (Klenow) were purchased from Takara Shuzo Co., Ltd. (Kyoto, Japan). DNA ligation
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was done according to procedures outlined by the manufacturer. The composition of media used was described (Miller, 1972). Synthesis of oligonucleotides was as described (Hidaka et al., 1988).

**Bacterial Strains, Plasmids, and Phages—** E. coli strains JM83 ara, Δlac-proA, rplB (=strA), 800 (lacZAM15) (Vieira and Messing, 1981) and JM25 harboring recA1, endA1, yqgA6, thi, hsdR17, supE44, relA1, Δlac-proAB, pBad108 (lactose inducible) and M13mplO (Vanishing Point, 1988) were used for host for plasmid and phage were taken from our laboratory stock. Plasmid pUC9 (Vieira and Messing, 1982) and phage M13mp10 (Messing, 1983) were also from our laboratory stock. pUC9-carrying AluI-HaeIII fragment (plasmid 134), on which a plasmid with a unique restriction site, terR1, was located, were as described (Horiuchi and Hidaka, 1988).

**DNA Preparation—** Plasmid and M13 replicative form DNA were isolated by the alkaline lysis procedure of Ish-Horowicz and Burke (1981). Some plasmid DNA were purified by equilibrium banding in CsCl gradients in the presence of ethidium bromide. Cells were transformed by the method of Kushner (1978). DNA fragments used for gel retardation assay were purified electrophoretically on acrylamide gels. The appropriate fragment was cut from the gel with ethidium bromide and obtained by the method using DEAE-paper from the gel (Drezen et al., 1981). Single-stranded M13 templates were prepared by the procedure of Nathans and Ekstein (1976). 

**Gel Retardation Assay—** Procedures used were essentially the same as described by Wang et al. (1987) and by our group (Kobayashi et al., 1989). As the DNA substrate for the assay, we used the 141-base pair AluI-HaeIII fragment, on which was located one of a pair of two terminus sites, terR1. The fragment was end-labeled with T4 polynucleotide kinase according to Maniatis et al., 1982.

**Construction of Ter Protein-overproducing Plasmid—** pUC9-5.0(–) was the parental plasmid used (Hidaka et al., 1988). The plasmid is a pUC derivative in the EcoRI site of which the 5.0-kilobase EcoRI fragment carrying terC2 site and the tau gene was inserted. A 2.7-kilobase HindIII-EcoRI subfragment of the 5.0-kilobase fragment was recloned into M13mp10 replicative form DNA, and the recombinant plasmid was used for the oligonucleotide-directed in vitro mutagenesis system, supplied by Amersham International plc. The mutagenesis procedures are based on the method of Eckstein and coworkers (Sayers et al., 1982). For footprinting, second mutant DNA was used, the oligonucleotides 5′-AATAAGATTTGTTAATCTAA-3′ (22-mer) and 5′-AATAAGATTTGTTACTAA-3′ (22-mer) were used, respectively. The nucleotide circled indicates each mutation point. Identification of the mutant protein was screened in hybridization experiments with the mutant oligonucleotide, the procedures of which were according to the method given by the supplier. Introduction of the first mutation was confirmed by DNA sequencing of the target site and surrounding areas. The replicative form DNA of the two mutant progeny phages was prepared, digested with HindIII and EcoRI, and recloned into the corresponding site of the pUC9 vector. The resulting two plasmids carrying single and double mutations (pM10) were used as the Ter protein overproducers.

**Overproduction and Purification of Ter Protein—** The Ter protein overproducer plasmid, pKHG300, was introduced into the JM83 host strain, and an ampicillin-resistant transformant was isolated. The method used for cell lysis preparation was essentially the same as described in Whitten et al. (1979). A fresh colony of the strain was inoculated into 50 ml of LB-ampicillin broth and shaken slowly at 300 rpm for 2 h, the centrifuged and precipitated fractions were dissolved in 500 μl of Buffer B, rplB (50 mM Tris-HCl (pH 7.5), 10 mM dithiothreitol, 20% glycerol) and ammonium sulfate powder was added to reach 90% saturation. After standing in an ice bath for 1 h, the precipitated fractions were dissolved in 500 μl of Buffer C (50 mM Tris-HCl (pH 7.5), 10 mM dithiothreitol, and 50% glycerol), and ammonium sulfate powder was added to reach a concentration that would achieve a 50% saturation. After standing in an ice bath for 1 h, the centrifuged and precipitated fractions were dissolved in 500 μl of Buffer D (50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 10% glycerol, and 50% ammonium sulfate), and the volumetric sodium acetate was added, 25 μl of Buffer D containing 0.5 mg/ml of Ter protein solution in dilution buffer (10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol) was applied to a DEAE-Sephacel (Pharmacia LKB Biotechnology Inc.) column (1 ml), and the flow-through fraction at 0.1 M NaCl (fraction I, 3 ml) was directly applied to a heparin-Sepharose CL-GB column (1 ml) (Pharmacia). The column was then washed with 4.5 column volumes of Buffer A containing 0.3 M NaCl and eluted sequentially with Buffer A containing 0.4, 0.5, 0.6, and 1.0 NaCl. Ter protein was eluted at 0.5–0.6 M NaCl (fraction IV, 3 ml).

**Protein Concentration Determination—** Protein concentration was determined using a BCA protein assay (Pierce) according to the method of Smith et al. (1985). Bovine serum albumin was used as the standard. Standards were prepared with the same concentration of each different ingredient of the assay kit. 

**DNA Sequence Analysis—** DNA sequences were carried out on a single-stranded M13 using the dideoxy chain termination method (Sanger et al., 1977). DNA sequence kits were obtained from United States Biochemical Corp. Appropriate 20-mer synthetic oligonucleotides were synthesized as additional primers for further sequencing along the same template.

**N1 Footprinting Assay—** The assay was performed according to the method of Galas and Schmitz (1978). The reaction mixture (100 μl) contained 10 μl of 10 × reaction buffer (100 mM Tris-HCl (pH 7.5), 500 mM NaCl, 10 mM dithiothreitol, 5 mM EDTA, 25 mM MgCl2, 50% glycerol); 2 μl of 7M end-labeled AluI (216-base pair) DNA fragment (~5.3 ng; 30 fmol); 10 μl (0.3–2 pmol) of Ter protein, and ammonium sulfate powder was added to reach 30% saturation. After incubating at 25 °C for 30 min, the action was terminated by adding 25 μl of stop solution (1.5 M sodium acetate (pH 5.2), 20 mM EDTA, 10 μg/ml sonicated calf thymus DNA). One hundred μl of phenol saturated with 100 mM Tris-HCl (pH 8.0) was added, Vortex mixed for 30 s, and centrifuged at 15,000 rpm. To the aqueous phase thus obtained we added 300 μl of ethanol, the preparation to stand at −70 °C for 30 min, and the pellicle was collected at 15,000 rpm for 4 °C and then dissolved with 100 μl of 0.3 M sodium acetate. After adding 250 μl of ethanol, the precipitation was repeated, the pellicle was washed twice with 300 μl of 70% ethanol and dried. Four μl of loading buffer (90% (v/v) deionized formamide, 50 mM Tris-HCl (pH 8.3), 0.1% (w/v) xylene cyanol, 0.1% (w/v) bromphenol blue, 1 mM EDTA) was added, 2 μl of the sample was applied to a polyacrylamide sequence gel and electrophoresed. As control samples, the same DNA fragment was used for basic-specific cleavage reactions, as described by Manas and Gilbert (1980), and electrophoresed. The gel was then dried and exposed to x-ray film (Kodak, X-Omat).

**Amino Acid and Amino-terminus Sequence Analysis—** Samples were hydrolyzed in tubes sealed under reduced pressure with 5.7 M HCl for 24, 48, and 72 h at 110 °C and with 3 M mercaptoethanesulfonic acid for 24 h at 110 °C. After evaporation, the hydrolysates were analyzed for amino acid analyzer (Shimadzu, 1958). Half-cystine was determined as cysteic acid after performic acid oxidation. Amino acid sequences were determined using an Applied Biosystems 477A gas-phase sequenator.

**RESULTS**

**Nucleotide Sequence of the tau Gene—** We had already identified the tau gene, defective mutants of which showed termination-less phenotype (Kobayashi et al., 1989). This gene was located on a 2.7-kilobase HindIII-EcoRI fragment situated at 35.5 min of the standard E. coli map (Bachmann, 1972; Kohara et al., 1987). Within or near the end of the tau gene was the terC2 site, one of the four DNA replication termination sites (terC) sites on E. coli genome (Hidaka et al., 1988; Hill et al., 1988b). The tau gene was localized within the 0.75–1.0-kilobase region from the terC2 site by insertional mutagenesis. The 2.7-kilobase HindIII-EcoRI fragment and its subfragment were cloned into the M13 family of vectors. Subsequent dideoxy sequencing of these clones revealed a single unique open reading frame in the region predicted to contain the tau gene, as shown in Fig. 1. In the promoter region of the gene, −35, −10, and ribosome-binding (Shine-Dalgarno) sequences were also present (Rosenberg and Court, 1979). Interestingly, the −10 region and ribosome-binding site overlapped with the terC2 sequence. The molecular weight of the open reading frame product was about 35,700. The sequence of the gene is exactly the same as that of the tau gene.
Fig. 1. Restriction map, sequencing strategy, and nucleotide and amino acid sequence of the tau gene. a, restriction map shows only relevant sites for EcoRI, HindIII, PvuII, and BglI. b. the underlined amino acids (without fMet) represent the amino-terminal sequence determined for the purified 36-kDa protein, ~35, ~10, and ribosomal-binding (Shine-Dalgarno) sites are underlined. The terC2 sequence is represented by bold letters. Two mutation changes, which convert the original tau plasmid to the Ter-overproducing plasmid, are shown.

reported by Hill et al. (1989) although there are differences in the region downstream of the tau gene.

Overproduction of the Ter Protein.—We observed that crude extracts of cells with the plasmid-carrying tau gene had about an 8-fold higher ter-binding activity than that seen in the plasmid-free cells (Kobayashi et al., 1989). To overproduce the protein, we introduced two mutations in vitro at the start site of the arrows shown as was the dieoxy nucleotide chain-terminating method (Sanger et al., 1977). The second mutation changes, which convert the original terC2 sequence is represented by bold letters. Two mutation changes, which convert the original tau plasmid to the Ter-overproducing plasmid, are shown.
two site-directed mutations

A

H

terC2

tau

lacZ

Amp<sup>r</sup>

ori

E

21034

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FIG. 2. Schematic structure of the Ter protein-overproducing plasmid (pKHG300). The 2.7-kilobase EcoRI-HindIII DNA fragment, on which the tau gene and terC2 site were located, was cloned into a polylinker site of the pUC9 vector plasmid. To overproduce Ter protein, two site-directed mutations, one within the terC2 sequence (E) and the other within the ribosomal-binding site of the tau gene, were introduced at the promoter site of the gene shown by the closed ellipse. Nucleotide changes of these mutations are shown in Fig. 1. This plasmid, named pKHG300, enabled the host cells to produce Ter protein over 2000-fold of the plasmid-free cells, even in the presence of higher amounts of lactose repressor. The long gray arrow indicates the coding region of the tau gene, and the short black arrow on the ori site represents direction of the replication fork beginning from the vector origin.

FIG. 3. SDS-polyacrylamide gel electrophoresis of 36-kDa Ter protein at different stages. Fraction I, crude extract, 9.83 μg; fraction II, ammonium sulfate precipitation, 38.85 μg; fraction III, after DEAE-Sephacel column chromatography, 1.42 μg; fraction IV, after heparin-Sepharose column chromatography, 0.82 μg; lane M, molecular standards (Pharmacia): phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20 kDa), and α-lactalbumin (14 kDa).

following the 36-kDa protein band through SDS-polyacrylamide gel electrophoresis analysis. Purification of the protein was attained by the procedures shown in Table I. The 36-kDa protein behaved as a basic protein; it did not bind to DEAE-Sephacel but did bind tightly to heparin-Sepharose, even in the presence of 0.1 M NaCl. Here we defined 1 unit of the ter-binding activity as that which could shift half of the terR1-containing DNA substrate (9.6 fmol) added to the band position corresponding to the DNA-protein complex, through the gel retardation assay, as shown in Fig. 4c. Using this enzyme unit, we determined the specific activity of the protein

TABLE I

| Fraction | Total protein (μg) | Total activity (units) | Specific activity (units/μg) |
|----------|-------------------|------------------------|-----------------------------|
| I. Lysate supernatant | 4912.5 | 145.5 × 10<sup>4</sup> | 296.2 (1.0) |
| II. Ammonium sulfate | 2033.6 | 142.0 × 10<sup>4</sup> | 698.3 (2.4) |
| III. DEAE-Sephadex | 708.0 | 50.4 × 10<sup>4</sup> | 711.9 (2.4) |
| IV. Heparin Sepharose CL-6B | 352.5 | 53.4 × 10<sup>4</sup> | 1514.9 (5.1) |

FIG. 4. Co-purification of ter-binding activity with the 36-kDa Ter protein through gel filtration. Sample (200 μl) of fraction IV was dialyzed with buffer (50 mM Tris-HCl (pH 9.0), 1 mM dithiothreitol, 1 mM EDTA, 5% (v/v) glycerol, 300 mM NaCl) overnight, applied to Superose 12HR 10/30 (Pharmacia), and eluted with the same buffer. Absorbance at 280 nm was measured with a UV detector, and a sample of the fraction (0.5 ml) was analyzed by SDS-polyacrylamide gel electrophoresis and gel retardation assay to measure the ter-binding activity. a, absorbance at 280 nm; b, SDS-polyacrylamide gel electrophoresis analysis; c, ter-binding activity. DNA substrate and DNA-protein complex indicate the positions corresponding to the substrate DNA fragment (141 base pairs) and DNA-protein complex, respectively.
fraction obtained at each stage of purification. Table I shows that the specific activity of ter-binding protein in the initial step was raised to 5-fold at the final step. This is near the expected level (7.14-fold). The 36-kDa protein in fraction IV, eluted from the heparin-Sepharose with 0.5-0.6 M NaCl, was further purified through gel filtration, and the ter-binding activities in each fraction were measured. As shown in Fig. 4, three different parameters (absorbance at 280 nm, a protein band of 36-kDa, and ter-binding activity) completely matched. We concluded that the 36-kDa protein is a Ter protein.

The amino-terminal amino acid sequence was determined for the purified 36-kDa protein in step IV using automated Edman degradation. Amino-terminal 45 residues, albeit with the interruption of 3 unidentified amino acids (including Met), is in perfect agreement with that deduced from the DNA sequence of the tau gene (Fig. 1). The amino acid composition (Table II) as well as the total molecular mass further supported the conclusion that the open reading frame, that is tau gene, encodes the Ter protein with a molecular mass of 36 kDa.

**DNA-binding Site of the Ter Protein**—To determine the binding site of the Ter protein, a DNase I footprinting experiment using purified Ter protein was done. Terminus sites (terR1 and terR2) of the plasmid R6K, essential for termination reaction, were located on 216 bp of AluI fragment (Horiuchi et al., 1987; Horiuchi and Hidaka, 1988). One end, closer to the terR2 site of the fragment, was labeled with 32P and mixed with the Ter protein, and partial DNase I digestion followed by polyacrylamide gel electrophoresis was performed. Fig. 5 shows that the Ter protein binds to the two sites that correspond exactly to terR1 and terR2. Since the sequences are highly homologous and placed in an inverted arrangement, two regions covered by the protein can be regarded as regions

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**TABLE II**

Amino acid composition of ter-binding protein

| Amino acid | Amino酸 composition predicted from DNA sequence |
|------------|-----------------------------------------------|
|            | DNA analysis†                               |
| Ala        | 27.8                                        |
| Arg        | 23.7                                        |
| Asn        | 29.2                                        |
| Asp        | 15.5                                        |
| Cys        | 15.8                                        |
| Gln        | 40.7                                        |
| Glu        | 22.2                                        |
| Gly        | 8.5                                         |
| His        | 15.6                                        |
| Ile        | 15.3                                         |
| Leu        | 39.3                                        |
| Lys        | 19.1                                        |
| Met        | 1.2                                         |
| Phe        | 8.5                                         |
| Pro        | 16.2                                        |
| Ser        | 11.0                                         |
| Thr        | 11.5                                         |
| Trp        | 4.3                                         |
| Tyr        | 7.9                                         |
| Val        | 22.5                                         |
| No. of residues | 308                                    |
| Calculated mass | 35,651                                |

† Average values obtained from 24-, 48-, and 72-h hydrolysis with 5.7 N HCl.

‡ Determined as cysteic acid.

§ Taken from 72-h values.

⁴ Extrapolated values to zero time.
of both DNA strands of either of the terR sites covered by the protein as shown in Fig. 6. The terR site was first identified as the 22-bp sequence required for the termination reaction in vivo and to which Ter protein binds.

**DISCUSSION**

We obtained convincing evidence that the tau gene encodes the ter-binding protein (Ter protein). This Ter protein recognized and bound to all ter sites (at least two terR and four terC sites), and the resulting Ter protein-ter sequence complex can block the DNA replication fork on the DNA molecule.

The sequence analysis of the tau gene suggested that there might be a site (terC2) at the promoter region of its own gene, to which Ter protein binds tightly. Since Ter protein overproduction was attained by a mutational destruction of the termination activity of the terC2 sequence, it is most probable that Ter protein might be a repressor for expression of its own gene; that is to say the tau gene is under autoregulatory control. The terC2 and -10 sequence apparently overlap, as shown in Fig. 1, and the overlapped sequence is covered by the Ter protein (Fig. 6). Thus, the binding of Ter protein to the terC2 site prevents RNA polymerase from interacting with the promoter site. This possibility was suggested by Hill et al. (1989). This autoregulatory control might maintain constant the quantity of the Ter protein. If such is indeed the case, then the Ter protein is exceptional; the protein plays two roles at the same site, terC2, one is a replication blocker, and the other is the repressor for its own gene expression.

However, with regard to Ter protein overproduction by the mutant plasmid, another explanation would have to be considered. As shown in Fig. 2, orientation of the replication fork starting from the pUC vector replication origin is that blocked by the terC2 site; the copy number of the parental plasmid is low. Mutational inactivation of the terC2 sequence resulted in reversion of its low copy number to normal level. Furthermore, orientation of the tau gene transcription is the same as that of lacZ gene on the vector plasmid. Thus, a high dosage of the tau gene and its high expression under lacZ control would make the gene product overproduce on the mutant plasmid. Evidence nonsupportive of this idea is that while the overproducing plasmid was introduced into JM109 (lacI^q; lacZ deletion mutant) the lacZ repressor-overproducing gene), the high level expression of the lacZ gene on the vector plasmid and with normal copy number was able to overproduce its product only 8-fold (Kobayashi et al., 1989). Thus, autoregulation seems to be a more tenable explanation. Hill et al. (1989) reported that the primary promoter of the tau (tau) gene is located at least 1200 base pairs upstream of the tau gene and that the weak promoter was identified immediately upstream of the tau gene. However, their results did not exclude the existence of the autoregulatory circuit.

In *B. subtilis*, a similar ter system seems to be operative. Wake and co-workers found that the terC site of *B. subtilis*, at which at a clockwise replication fork was blocked, was located at the region opposite to that of the origin of replication (Weiss and Wake, 1983, 1984). Sequence analysis of the region revealed that at the terC site, there is a long inverted repeat sequence homologous to the *E. coli* terC sequence (Carrigan et al., 1987). Adjacent to the terC site, there is an open reading frame capable of coding a basic protein with 122 amino acids, the defective mutant of which showed a termination-less phenotype (Iismaa and Wake, 1987; Smith and Wake, 1988; Lewis and Wake, 1989). Recently this protein, like the *E. coli* tau product, was found to have terC-binding activity (Lewis et al., 1989), although they apparently share no homologous region (Hill et al., 1989).

Sista et al. (1989) purified the terR-binding protein of *E. coli* and determined the binding site of the purified protein. This protein may be the same as the one purified in our present study; however, the region covered by their protein is narrower than that shown in our Fig. 6, although both patterns are essentially the same. The different techniques used may account for the discrepancies; we used DNa I and they used copper-phenanthroline (Kuwabara and Sigman, 1987) for the footprinting experiment.

The molecular structure of the Ter protein-ter sequence complex seems to be unique since the Ter protein is the first example of a DNA-binding protein that can block movement of the DNA replication fork. Particulars regarding this block and the molecular mechanisms involved in progress of the replication fork at the replication point are now being investigated.

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