JOURNAL OF BACTERIOLOGY, Jan. 1993, p. 277–287
0021-9193/93/010277-11$02.00/0

Vol. 175, No. 1

Identification and Characterization of the *Escherichia coli* RecT Protein, a Protein Encoded by the *recE* Region That Promotes Renaturation of Homologous Single-Stranded DNA

SHARYNN D. HALL, MICHAEL F. KANE, AND RICHARD D. KOLODNER*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, and Division of Cellular and Molecular Biology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115*

Received 7 May 1992/Accepted 12 September 1992

Recombination of plasmid DNAs and recombination of bacteriophage λ *red* mutants in *recB recC sbcA Escherichia coli* mutants, in which the *recE* region is expressed, do not require *recA*. The *recE* gene is known to encode exonuclease VIII (exoVIII), which is an ATP-independent exonuclease involved in the RecE pathway of recombination. A 33,000-molecular-weight (MW) protein was observed to be coexpressed with both exoVIII and a truncated version of exoVIII, pRac3 exo, when they were overproduced under the control of strong promoters. We have purified this 33,000-MW protein (p33) and demonstrated by protein sequence analysis that it is encoded by the same coding sequence that encodes the C-terminal 33,000-MW portion of exoVIII. p33 is expressed independently of exoVIII but is probably translated from the same mRNA. p33 was found to bind to single-stranded DNA and also to promote the renaturation of complementary single-stranded DNA. It appears that p33 is functionally analogous to the bacteriophage λ β protein, which may explain why RecE pathway recombination does not require *recA*.

Genetic analysis of recombination in *Escherichia coli* has demonstrated the existence of several pathways of recombination. Initial studies identified RecBCD and RecA as important components of the major recombination pathway that functions in wild-type *E. coli* cells (33, 48). Alternative recombination pathways in *E. coli* were subsequently identified through the isolation and analysis of mutations that suppressed *recB recC* double mutants (1, 50). Historically, recombination occurring in *recB recC* mutants containing *sbcA* suppressor mutations was called RecE pathway recombination, and recombination occurring in *recB recC* mutants containing *sbcB sbcC* suppressor mutations was called RecF pathway recombination (5–7, 17, 26). However, this view of recombination pathways is an oversimplification, because the exact gene products required for recombination appear to depend on both the structure of the recombining DNA molecules and the gene products present in the cell (6, 10, 13, 23, 27, 28, 30, 32, 34, 49).

Recombination in *recB recC sbcA* mutants has proven to be particularly complicated. *sbcA* mutations activate the expression of the *recE* region encoded by the Rac lambda-like cryptic prophage (1, 8, 11, 13, 50). There is a possibility that *sbcA* mutations also activate the expression of genes other than *recE* whose products act in recombination. In *recB recC sbcA* mutants, all recombination events thus far examined appear to require the *recE* gene product (7, 8, 11, 13, 32, 49). The other gene products required are determined in part by the structure of the recombination substrate. Recombination after conjugation has been shown to require *recE*, *recA*, and the RecF pathway genes *recF*, *recJ*, *recN*, *recO*, *recQ*, *recR*, *ruvA*, *ruvB*, and *ruvC* and, in our view, appears to actually be promoted by the RecF pathway or a RecF-like pathway (13, 27, 29, 32, 35, 49). Recombination after P1 transduction is similar to conjugational recombination (8). Circular plasmid recombination requires *recE* and a subset of RecF pathway gene products, those of *recF*, *recJ*, *recO*, *recQ*, *recR*, and *ruvB*, but does not require *recA* (3, 10, 18, 23, 32). The effects of *ruvA* and *ruvC* mutations on plasmid recombination have not yet been tested in this genetic background. In contrast, recombination of linear dimer plasmids and recombination of λ *redα redβ* require only *recE* and do not appear to require *recA* or any other known *E. coli* recombination gene products, such as those encoded by the RecF pathway genes (3, 11, 13, 32, 46, 49, 51). The effects of all known RecF pathway mutations on λ *red* recombination have not yet been evaluated in the *sbcA* background. Sawitzke and Stahl (46) recently characterized the effect of RecF pathway mutations on λ phage recombination and found that phage carrying a deletion of the *nin5* region require the *E. coli recO*, *recR*, and *recF* gene products during recombination. It appears that λ encodes a *recORF* analog that substitutes for the requirement of *recF*, *recO*, and *recR* when λ *red* phage recombine by the RecF pathway. However, neither the *recORF* analog nor the *recF*, *recO*, or *recA* gene product appears to be required for λ phage recombination by the Red pathway or by the RecE pathway that functions in *recB recC sbcA* mutants. Our interpretation of these observations suggests that two different recombination pathways are activated by *sbcA* mutations, the RecF pathway, which also requires the *recE* gene product under these circumstances, and a completely different, *recA*-independent pathway that we refer to as the RecE pathway.

The *recE* gene has been shown to encode an ATP-independent exonuclease called exonuclease VIII (exoVIII) (22). This exonuclease is a 140,000-M_r polypeptide that exists as a tetramer in solution (19, 20). It degrades linear duplex DNA in the 5′-to-3′ direction, yielding 5′-mononucleotides as products. It has a strong preference for linear duplex substrate DNA and appears to be unable to initiate degradation from single-strand breaks in DNA (20). One of

* Corresponding author.

the striking features of exoVIII is its high processivity, in that it is capable of degrading DNA on the order of 20,000 bases per initiation event (15). Biochemically, exoVIII resembles λ exo, the product of the *redα* gene (12, 24, 25, 42). Genetic evidence supports these biochemical similarities. First, *sbcA* mutations, which activate the expression of exoVIII, are able to complement *red* mutations (11, 13). Second, in λ reverse phage, which originally lacked the recombination functions encoded by λ, a region of the phage genome has been replaced with a portion of the *E. coli*-encoded Rac prophage, which encodes exoVIII, and this replacement allows them to grow under conditions in which recombination is essential (12, 14). This substitution renders these phage recombination proficient. These results demonstrate that exoVIII can substitute for λ exo in λ recombination. However, exoVIII and λ exo differ in size, are antigenically distinct, and do not share any sequence homology (4, 14, 19, 22, 43).

Other genetic evidence suggests that the *recE* region encodes at least one protein in addition to exoVIII that is required for recombination. First, the expression of *recE* substitutes for two bacteriophage λ-encoded proteins required for recombination: λ exo, encoded by the *redα* gene, and the *redβ* protein, which is a single-stranded DNA binding protein that can promote renaturation of DNA and stimulate RecA-dependent strand exchange in vitro (12, 14, 21, 39, 41, 42, 44). Second, some recombination events in *recB recC sbcA* mutants do not require *recA*, a fact suggesting that a second homologous pairing protein may exist and substitute for RecA (10, 18, 23, 32, 49). This idea is consistent with the observation that the λ β protein is able to substitute for RecA under some circumstances (2) and suggests that the *recE* region may encode a λ β protein analog. Genetic studies (unpublished data) suggest that the *recE* region encodes two distinct functions required for recombination and repair in *recB recC sbcA* mutants (9). It appears that the DNA sequence of the *recE* region can direct the synthesis of two proteins: exoVIII, which is likely produced by a translational frameshift event (9), and possibly a protein produced by translational reinitiation (9; this work). In this communication, we demonstrate that, in addition to exoVIII, the *recE* region encodes a 33,000-M_r protein that binds to single-stranded DNA and, like the λ β protein, promotes renaturation of DNA.

MATERIALS AND METHODS

Strains, plasmids, and bacteriological techniques. *E. coli* JC5519 (*recB21 recC22 his-4 argE3 leuB6 proA2 thr-1 thi-1 rpsL31 galK2 lacY1 ara-14 xyl-5 mtl-1 kdgK51 supE44 tsx-33*) containing pRac31 or pRac3 was obtained from A. J. Clark (University of California, Berkeley). pRac31 is pBR322 which has inserted into its *Hin*dIII site a 7.6-kb *Hin*dIII fragment derived from Rac, which carries a functional wild-type *recE* gene. pRac3 is a similar plasmid, except that the inserted *Hin*dIII fragment is a 5.7-kb fragment containing a deletion mutation fusing the C-terminal portion of *recE* and the N-terminal region of an upstream gene, *racC*, resulting in the expression of a fusion protein(s) that suppresses *recB recC* mutations like *sbcA* mutations. These plasmids have been described previously (4, 55). RG1, a derivative of pACYC177 that contains the *lacI*^{q} gene, was obtained from Robert Garcia (Dana-Farber Cancer Institute). pGP1, a pBR322-based plasmid containing both the gene encoding T7 RNA polymerase under the control of lambda p_L and the lambda *c*I857 gene, and pT7-5, a T7 expression vector, were obtained from Stan Tabor (Harvard Medical School). *E. coli* RDK1400 (*thr leuB6 thi thyA trpC1117 hsrK12 hsmK12 str recA13*) and pUC19 were from our laboratory collection and were used for most cloning and expression experiments. Unless indicated, all cultures were grown in L broth supplemented with 50 μg of thymine, 50 μg of ampicillin, and/or 30 μg of kanamycin per ml when applicable. Transformations were carried out as described elsewhere (52).

Chemicals. PBE94, Superose-6, and the high- and low-molecular-weight protein gel filtration standards were from Pharmacia LKB Biotechnology (Piscataway, N.J.). Hydroxylapatite and sodium dodecyl sulfate ($NaDodSO_4$)-polyacrylamide gel electrophoresis (PAGE) molecular weight standards were from Bio-Rad Laboratories (Richmond, Calif.). Isopropyl-β-D-thiogalactopyranoside (IPTG), rifampin, salmon sperm DNA, spermidine HCl, and amino acids were from Sigma (St. Louis, Mo.). [^{35}S]methionine was from Amersham (Arlington Heights, Ill.), and [^{3}H]thymidine was from DuPont-New England Nuclear (Boston, Mass.). BA85 nitrocellulose filters and GF/C glass fiber filters were from Schleicher & Schuell (Keene, N.H.).

Enzymes. All restriction endonucleases were from New England BioLabs (Beverly, Mass.) and were used under the reaction conditions suggested by the supplier. S1 nuclease was from BRL (Bethesda, Md.). The purification of exoVIII will be described elsewhere (15). pRac3 exo and p33 were purified as described below.

DNA substrates. Unlabelled T7 DNA and ^{3}H-T7 DNA, which had a specific activity of 21,670 cpm/nmol, were purified as described previously (45). ^{3}H-M13mp19 replicative-form (RF) DNA (5,280 cpm/nmol), ^{3}H-single-stranded viral DNA (3,795 cpm/nmol), and unlabelled M13mp19 RF and viral DNAs were prepared as described previously (40).

Construction of plasmids and overproducing strains. Plasmids pRDK229 and pRDK230 were constructed by inserting the 5.7-kb *Hin*dIII fragment from pRac3 and the 7.6-kb fragment from pRac31, respectively, into the *Hin*dIII site of pUC19 in the orientation required for transcription from the *lacZ* promoter. These plasmids were transformed into RDK1400 containing RG1 to yield strains RDK2061 and RDK1992, respectively. Two additional plasmids, pRDK260 and pRDK262, were similarly constructed by inserting the 5.7- and 7.6-kb *Hin*dIII fragments from pRDK229 and pRDK230, respectively, into the unique *Hin*dIII site of pT7-5 in the orientation required for transcription from the T7 promoter. These plasmids were transformed into RDK1400 containing pGP1 to yield strains RDK2678 and RDK2679, respectively.

Exonuclease assay. Standard exonuclease assay mixtures (100 μl) contained 20 mM Tris (pH 8.0), 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, 2.5 nmol of ^{3}H-double-stranded T7 DNA, and enzyme. After incubation for 10 min at 37°C, each reaction was stopped by the addition of 100 μl of ice-cold H_2O, 300 μl of 0.22-mg/ml salmon sperm DNA, and 300 μl of 1 N trichloroacetic acid. The samples were placed on ice for 5 min and then centrifuged for 15 min in a microcentrifuge at 4°C. A 400-μl quantity of the supernatant was removed and added to 4 ml of Ready Safe scintillation fluor (Beckman, Fullerton, Calif.), and the radioactivity present was determined by scintillation counting. One unit of exonuclease activity is the amount of enzyme required to render 1 nmol of double-stranded DNA acid soluble in 10 min at 37°C. All reactions were performed in duplicate.

DNA binding assay. Standard assay reaction mixtures (30 μl) contained 33 mM Tris (pH 7.5), 13 mM $MgCl_2$, 1.8 mM

dithiothreitol, 88 μg of bovine serum albumin per ml, 400 ng of ^{3}H-single-stranded M13mp19 viral DNA, and enzyme. After incubation for 10 min at 37°C, each reaction mixture was diluted with 500 μl of wash buffer containing 33 mM Tris (pH 7.5), 13 mM $MgCl_2$, and 1.8 mM dithiothreitol and then filtered through KOH-treated nitrocellulose membranes (38) by use of a manifold filtration unit (Millipore, Bedford, Mass.). Each filter was washed once with wash buffer and dried. The amount of radioactivity bound to each filter was then determined by scintillation counting. Each reaction was performed in duplicate.

Renaturation assays. The formation of renatured products from heat-denatured single-stranded DNA was assayed by both agarose gel electrophoresis and S1 nuclease resistance assays. For the agarose gel electrophoresis assay, each reaction mixture (30 μl) contained 33 mM Tris (pH 7.5), 13 mM $MgCl_2$, 1.5 mM dithiothreitol, 100 mM KCl, 500 ng of heat-denatured T7 DNA, and p33 as indicated in individual experiments. After 10 min of incubation at 37°C, 0.5 M EDTA (pH 8.0), 1.0% $NaDodSO_4$ and 20 mg of proteinase K per ml were added to final concentrations of 50 mM, 0.1%, and 530 μg/ml, respectively, and incubation was continued for an additional 10 min at 37°C. Three microliters of running dye (0.25% bromophenol blue, 0.25% xylene cyanol FF, 15% Ficoll) was added, and the samples were loaded onto an 0.8% agarose gel, which was then run in buffer containing 40 mM Tris-acetate (pH 7.9), 1 mM EDTA, and 0.5 μg of ethidium bromide per ml and photographed. Reaction mixtures containing single-stranded M13mp19 viral substrate DNA were incubated for 10 min at 37°C under the same reaction conditions and contained p33 or *E. coli* single-stranded DNA binding protein (SSB) as indicated in individual experiments. These reactions were terminated by the addition of 0.5 M EDTA and, as indicated in individual experiments, 1% $NaDodSO_4$ and 20 mg of proteinase K per ml to the same final concentrations as those indicated above, and then the reaction mixtures were incubated for an additional 10 min at 37°C. S1 nuclease assays were performed in duplicate with 500 ng of heat-denatured ^{3}H-T7 DNA or 400 ng of ^{3}H-M13mp19 viral DNA as a substrate essentially as described previously (16). Reaction conditions and volumes were those described above for the agarose gel electrophoresis assay. After incubation of the product DNA with S1 nuclease, an equal volume of 1 N trichloroacetic acid was added and the amount of acid-precipitable DNA present was determined by scintillation counting essentially as described above.

Purification of pRac3 exo and p33. Purification of pRac3 exo was done by a modification of a previously published procedure (31) and monitored by both $NaDodSO_4$-PAGE and exonuclease assays. p33 purification was monitored by $NaDodSO_4$-PAGE. Unless otherwise indicated, all procedures were performed at 0 to 4°C. Logarithmic-phase cultures (optical density at 600 nm = 0.5 to 0.7) of RDK2061 grown at 37°C were induced by the addition of IPTG to a final concentration of 1 mM and grown for an additional 4 h. The cells were harvested by centrifugation for 10 min at 7,000 rpm in a GSA rotor by use of a Sorvall RC-5 centrifuge. The cells were resuspended in 10% (wt/vol) sucrose–50 mM Tris-HCl (pH 7.5) to a final volume of 20 ml/liter of original cells, and the suspension was frozen in liquid N_2 and stored at −75°C. A 120-ml quantity of frozen cells was thawed on ice. Then, 5 M NaCl, 0.5 M spermidine, and 10 mg of lysozyme per ml in 10% (wt/vol) sucrose–50 mM Tris-HCl (pH 7.5) were added to a final concentrations of 100 mM, 10 mM, and 0.2 mg/ml, respectively, and the resulting lysis mixture was incubated on ice for 45 min. The cells were heated from 0 to 20°C by incubation in a 37°C water bath and then cooled on ice until they reached 10°C. The extract was centrifuged at 13,500 rpm in a Sorvall SA600 rotor for 30 min, and the supernatant was saved (fraction I; 97 ml). Solid ammonium sulfate (0.176 g/ml) was added to fraction I with stirring on ice over a 30-min period. After an additional 60 min of stirring, the solution was centrifuged at 13,000 rpm in a Sorvall SA600 rotor. The pellet was resuspended with buffer A (20 mM Tris [pH 7.5], 10 mM 2-mercaptoethanol, 0.1 mM EDTA, 10% [wt/vol] glycerol) containing 100 mM NaCl to yield a final volume of 20 ml (fraction II). Fraction II was diluted with buffer A to obtain a conductivity equivalent to that of buffer A containing 100 mM NaCl and loaded at 25 ml/h into a PBE94 column (20 by 1.5 cm) that had previously been equilibrated with buffer A containing 100 mM NaCl. The column was washed with an additional 200 ml of buffer A containing 100 mM NaCl, and the proteins were eluted with a 700-ml linear gradient of buffer A containing 100 to 1,000 mM NaCl. The 42.5-kDa pRac3 exo eluted at between 500 and 650 mM NaCl. The fractions containing pRac3 exo were pooled (35 ml) and dialyzed against buffer A containing 60% (wt/vol) glycerol (instead of 10% [wt/vol] glycerol) and 100 mM NaCl to yield a final volume of 14 ml (fraction III), which was stored at −20°C. p33 eluted from the PBE94 column at between 200 and 400 mM NaCl, and the fractions were pooled to yield p33 fraction IV (42.5 ml). Thirty milliliters of p33 fraction IV was adjusted to a final concentration of 50 mM KPO_4 (pH 7.5) by the addition of 2 M KPO_4 (pH 7.5) and loaded at 25 ml/h into a hydroxylapatite column (17 by 1.5 cm) that had previously been equilibrated with buffer B (50 mM KPO_4 [pH 7.5], 10% [wt/vol] glycerol, 10 mM 2-mercaptoethanol). The column was washed with an additional 500 ml of this buffer, and the proteins were eluted with a 627-ml linear gradient of buffer B containing 50 mM to 1 M KPO_4 (pH 7.5). p33 eluted at between 200 and 400 mM KPO_4, and the fractions were pooled and dialyzed against buffer A containing 60% (wt/vol) glycerol (instead of 10% [wt/vol] glycerol) and 100 mM NaCl to yield p33 fraction IV (28 ml), which was stored at −20°C.

Protein analysis. $NaDodSO_4$-PAGE was used to monitor both the synthesis and the purity of pRac3 exo and p33 (36). For the analysis of protein present in whole cells, 1 ml of logarithmic-phase cells (optical density at 600 nm = 0.5 to 0.7) was pelleted in a microcentrifuge and resuspended in 100 μl of 1× $NaDodSO_4$ sample solution, which contained 50 mM Tris-HCl (pH 7.0), 2% (wt/vol) $NaDodSO_4$, 5% (vol/vol) 2-mercaptoethanol, 0.005% (wt/vol) bromophenol blue, and 5% (wt/vol) sucrose (36). Samples were then incubated at 100°C for 2 to 3 min, and 10 to 15 μl of each sample was analyzed by electrophoresis on a 12% $NaDodSO_4$-polyacrylamide gel and stained with Coomassie blue. For analysis of other protein fractions, samples were mixed with an equal volume of 2× $NaDodSO_4$ sample solution and then treated as described above.

For protein sequence analysis of p33, fraction IV was further purified by preparative $NaDodSO_4$-PAGE, electroblotted onto a polyvinylidene difluoride membrane, and stained as described previously (37). The band corresponding to the p33 protein was excised and stored at −20°C. Twelve amino acids from the N terminus of the 33-kDa protein were then sequenced by Ruth Steinbrich (Laboratory of Immunobiology, Dana-Farber Cancer Institute) by use of an Applied Biosystems model 470A sequencer.

For pulse-chase analysis of the synthesis of p33, pRac3 exo, and exoVIII, overnight cultures of RDK2678 and

RDK2679 were grown at 30°C in L broth supplemented with 0.2% glucose, 50 μg of ampicillin per ml, and 30 μg of kanamycin per ml. One milliliter of each culture was diluted into 10 ml of L broth plus supplements and grown to an optical density at 600 nm of 0.5. Ten milliliters of cells was pelleted by centrifugation in a Sorvall T6000B tabletop centrifuge (Dupont, Wilmington, Del.) for 3 to 5 min at 5,000 rpm and room temperature, washed by resuspension in 5 ml of 56/2 minimal salts medium (53) supplemented with 50 μg each of thymine, thiamine, tryptophan, leucine, and threonine per ml and 0.2% glucose, and pelleted again by centrifugation. The cell pellet was resuspended in 5 ml of 56/2 minimal salts medium with supplements and grown for an additional 60 min at 30°C. A 3.5-ml quantity of each culture was heat induced by being shaken at 42°C for 15 min and grown for an additional 10 min at 42°C in the presence of 200 μg of rifampin per ml. The temperature was shifted to 40°C for 20 min, after which time 10 μCi of [^{35}S]methionine was added to each culture, and incubation at 40°C was continued for 3 min. The cells were pelleted as described above, washed twice by resuspension in 56/2 minimal salts medium with supplements and 0.1% methionine, and finally resuspended in 3 ml of this medium. Aliquots (500 μl) were removed at various times, pelleted, resuspended in 50 μl of 1× NaDodSO$_4$ sample solution, and stored frozen at −75°C overnight. For NaDodSO$_4$-PAGE analysis, the samples were thawed and incubated for 3 to 5 min at 100°C, and then 10 μl of each sample was loaded onto a 12% polyacrylamide gel. The gel was run at a constant current of 30 mV, stained, destained, dried, and exposed overnight on Kodak XAR film or a Phosphorimager screen (Molecular Dynamics, Sunnyvale, Calif.). A Phosphorimager II machine was used to quantitate the relative radioactivity in each band.

Sedimentation coefficients were determined by centrifugation of protein samples and standards through 5-ml 20 to 40% (wt/vol) linear glycerol gradients in a buffer containing 20 mM Tris-HCl (pH 7.5), 10 mM 2-mercaptoethanol, 0.1 mM EDTA, and 200 mM NaCl. Samples were centrifuged at 45,000 rpm for 19 h at 4°C in a Beckman (Fullerton, Calif.) SW55 rotor, and fractions were collected by use of a peristaltic pump (Pharmacia). The proteins were detected by a Bradford protein assay or on the basis of enzymatic activity. The sedimentation coefficients of p33, pRac3 exo, and exoVIII were determined by comparison with standards having known S values. The standards used were bovine serum albumin (4.3S), aldolase (7.3S), catalase (11.3S), and ferritin (17.5S). The Stoke's radius was determined by gel filtration controlled by a Waters (Milford, Mass.) 650E advanced protein purification system. Bovine serum albumin (35.5 Å [1 Å = 0.1 nm]), aldolase (48.1 Å), catalase (52.2 Å), ferritin (61 Å), thyroglobulin (85 Å), p33, pRac3 exo, and exoVIII were chromatogaphed on a Superose-6 column (8.5 by 0.52 cm) run at 50 μl/min with buffer A containing either 200 mM NaCl or 1 M NaCl, and the elution of each protein was monitored by measuring the A_{280}. The elution volume (v_e) for each sample was calculated as the time of the peak absorbance × the rate at which the column was run. K_{av}, the fractional retention of a sample, was calculated by use of the equation $K_{av} = (v_e - v_o)/(v_t - v_o)$, where v_o is the void volume determined by use of T7 phage as the standard and v_t is the total volume of the column. $-(\log K_{av})^{1/2}$ was calculated and plotted against the Stoke's radius (in angstroms) of known standards b the method of Siegel and Monty (47). The Stoke's radii of p33, pRac3 exo, and exoVIII were determined by use of this standard curve.

Protein concentrations were determined by Bradford analysis with reagents obtained from Bio-Rad and bovine serum albumin as a standard.

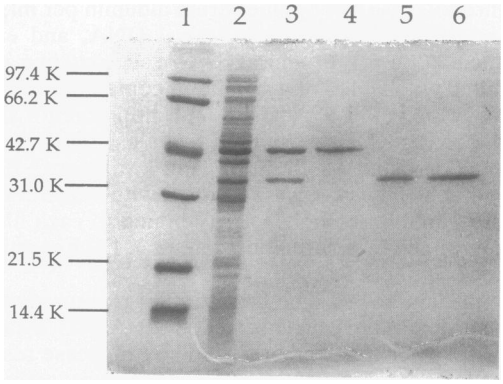

FIG. 1. NaDodSO$_4$-PAGE analysis of the purification of pRac3 exo and p33. pRac3 exo and p33 were purified and analyzed by NaDodSO$_4$-PAGE as described in Materials and Methods. Lanes: 1, molecular weight markers; 2, fraction I, 15 μg; 3, fraction II, 4 μg; 4, fraction III, pRac3 exo, 0.6 μg; 5, fraction III, p33, 0.5 μg; 6, fraction IV, p33, 0.5 μg.

RESULTS

Purification of pRac3 exo and p33. The 42.5-kDa pRac3 exo is a truncated form of exoVIII (4, 8, 55) that was previously identified by Western blotting (immunoblotting) analysis with polyclonal antibodies directed against exoVIII and purified (31). We overproduced pRac3 exo under the control of a *lacZ* promoter inducible by IPTG. pRG1, which carries the *lacI*^{q} gene, was maintained in RDK2061 to prevent lethal overexpression of pRac3 exo prior to induction with IPTG. pRac3 exo was purified to homogeneity by precipitation with ammonium sulfate and chromatography on PBE94 (Fig. 1 and Table 1) by a modification of a previously published procedure (31). Analysis by NaDodSO$_4$-PAGE indicated that the final fraction was greater than 99% pure (Fig. 1, lane 4). A 33-kDa protein appeared to be coexpressed with pRac3 exo after induction with IPTG (Fig. 1, lane 2) and also to be copurified with pRac3 exo through the ammonium sulfate fractionation step (Fig. 1, lane 3). The 33-kDa protein (p33) was purified to near homogeneity by sequential chromatography on PBE94 and hydroxylapatite (Table 1 and Fig. 1). p33 was also detected during overexpression of the full-length exoVIII (data not shown) and verified to have the

TABLE 1. Purification of pRac3 exo and p33 from a pRac3 exo-overproducing strain[a]

Fraction	pRac3 exo				p33 total protein (mg)
	Total protein (mg)	Activity (10^6 U[b])	Sp act (U/mg)	Yield (%)	
I (extract)	223	9.7	43,400	100	223
II (ammonium sulfate)	150	47.9	319,000	494	150
III (PBE94)	73.5	18.2	248,000	188	54.8
IV (hydroxylapatite)					53.7

[a] pRac3 exo and p33 were purified from 6 liters of IPTG-induced RDK2062 cells exactly as described in Materials and Methods.

[b] One unit is the amount of enzyme causing the production of 1.0 nmol of acid-soluble material in 10 min under standard assay conditions.

```
CTA AGG AAT ATG CAA ATG ACT AAG CAA CCA CCA ATC GCA AAA GCC GAT CTG CAA
    RBS           M   T   K   Q   P   P   I   A   K   A
```

FIG. 2. N-terminal protein sequence of p33. The top line shows the DNA sequence of the *recE* ORF from nucleotides 2578 to 2631 (9). The bottom line shows the N-terminal protein sequence obtained from sequencing of p33 as described in Materials and Methods. A potential ribosome binding site within the *recE* ORF is underlined.

same N-terminal amino acid sequence as p33 purified from pRac3 exo-overpressing strains.

Identification of p33. Amino-terminal sequence analysis of p33 purified from both the pRac3 exo- and the exoVIII-overproducing strains yielded MTKQPPIAKA, which exactly matched a region of predicted protein sequence encoded by the *recE* open reading frame (ORF) (9) (Fig. 2). The N-terminal Met of p33 mapped to a codon at nucleotide position 2593 of the exoVIII ORF (numbered from the initial Met of exoVIII). This Met codon is the first Met codon present in the *recT* ORF identified by Clark et al. (9). The region of this ORF starting at the Met codon and extending to the first termination codon is predicted to encode a 29.5-kDa polypeptide, which closely matches the size of p33. This analysis indicates that p33 is encoded by the *recT* ORF. Further analysis of the DNA sequence revealed a probable ribosome binding site at an optimal distance away from the initial Met of p33 (Fig. 2). This result suggests that the 33-kDa protein is encoded by the same mRNA as exoVIII but is independently translated from this mRNA. An alternate possibility is that p33 is processed from exoVIII and pRac3 exo by proteolytic cleavage. However, pulse-chase analysis (described below) indicates that this is an unlikely possibility.

To determine the relationship between p33 and pRac3 exo or exoVIII, we analyzed the expression of these proteins by pulse-chase analysis with plasmids that expressed either the pRac3 region or the intact *recE* region (containing the structural gene of exoVIII) under the control of a T7 promoter. This system allowed the exclusive analysis of plasmid-encoded proteins. p33 and pRac3 exo were present at constant levels after the initial pulse with [^{35}S]methionine and throughout 60 min of chase (Fig. 3). p33 and full-length exoVIII were also present at relatively constant levels after the initial pulse and throughout 60 min of chase. We believe the modest increase in the ratio of p33 to exoVIII at 60 min was due to the general degradation of exoVIII, which is a large protein. In both experiments, there was no additional incorporation of [^{35}S]methionine into p33, pRac3 exo, or exoVIII after the initial pulse, a result indicating that the methionine chase was effective. This analysis indicates that p33 was not processed from either exoVIII or pRac3 exo but rather was cotranslated with these proteins. Figure 3 shows the molar ratios of p33 to pRac3 exo and exoVIII during the chase period. To determine the expression of p33 relative to that of pRac3 exo or exoVIII, we estimated the number of methionine residues present in pRac3 exo from its size and sequence relationship with the *recE* gene (4, 9) and calculated the number of methionine residues present in p33 and exoVIII from their predicted sequences by assuming that exoVIII results from a translational frameshift, as predicted by Clark and coworkers (9). Constant molar ratios of 3:1 and 10:1 were obtained for the expression of p33 relative to those of pRac3 exo and exoVIII, respectively.

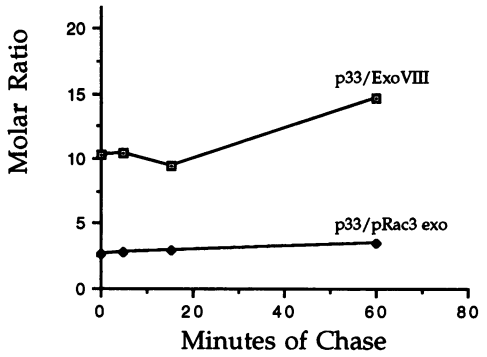

FIG. 3. Pulse-chase analysis of the expression of p33, exoVIII, and pRac3 exo. The expression of p33 relative to that of either exoVIII or pRac3 exo was measured by pulse-chase analysis as described in Materials and Methods, and the relative amount of radioactivity present in each protein species was determined by densitometry of the resulting autoradiograms. The molar ratios of p33 relative to pRac3 exo and exoVIII during the chase period were calculated from the estimated number of methionines in each protein.

Physical characterization of pRac3 exo and p33. The sizes of the pRac3 exo, p33, and exoVIII polypeptides were estimated by NaDodSO$_4$-PAGE to be 42.5, 32.5, and 140 kDa, respectively (8, 31; this work). exoVIII appears to migrate anomalously during analysis by NaDodSO$_4$-PAGE, since the *recE* and *recT* DNA sequences predict that exoVIII should be a 126-kDa protein (4, 9). The native molecular weights of pRac3 exo and p33 were calculated from the Stoke's radii determined by gel filtration and the sedimentation coefficients determined by glycerol gradient sedimentation (47) (Fig. 4). These values are summarized in Table 2 and suggest that pRac3 exo and p33 are a hexamer and a tetramer, respectively.

DNA binding properties of p33. The *recE* coding region expresses proteins capable of substituting for both the bacteriophage λ β and the bacteriophage λ exo proteins. p33 is a logical candidate for the protein that substitutes for β protein, an idea suggesting that it may have biochemical properties that are similar to the properties of β protein. Therefore, the ability of p33 to bind to single-stranded DNA and also to promote the renaturation of homologous single-stranded DNA was investigated.

DNA binding was characterized by measuring the ability of p33 to form stable protein-DNA complexes that could be trapped on nitrocellulose filters. The formation of protein-DNA complexes was not detected when linear double-stranded M13mp19 RF DNA was used as a substrate. However, the formation of protein-DNA complexes was readily detected when single-stranded M13mp19 viral DNA was used as the substrate (Fig. 5). One-half maximal binding was observed at a protein/DNA ratio of 362 p33 monomers (90 tetramers) per DNA molecule (Fig. 5). Complex formation was rapid and was complete by the time a reaction mixture could be prepared at 0°C and filtered (data not shown). The reaction requirements for binding are summarized in Table 3. Binding did not appear to have a large requirement for $MgCl_2$ or reducing agents, such as dithiothreitol. Complex formation was only slightly inhibited by

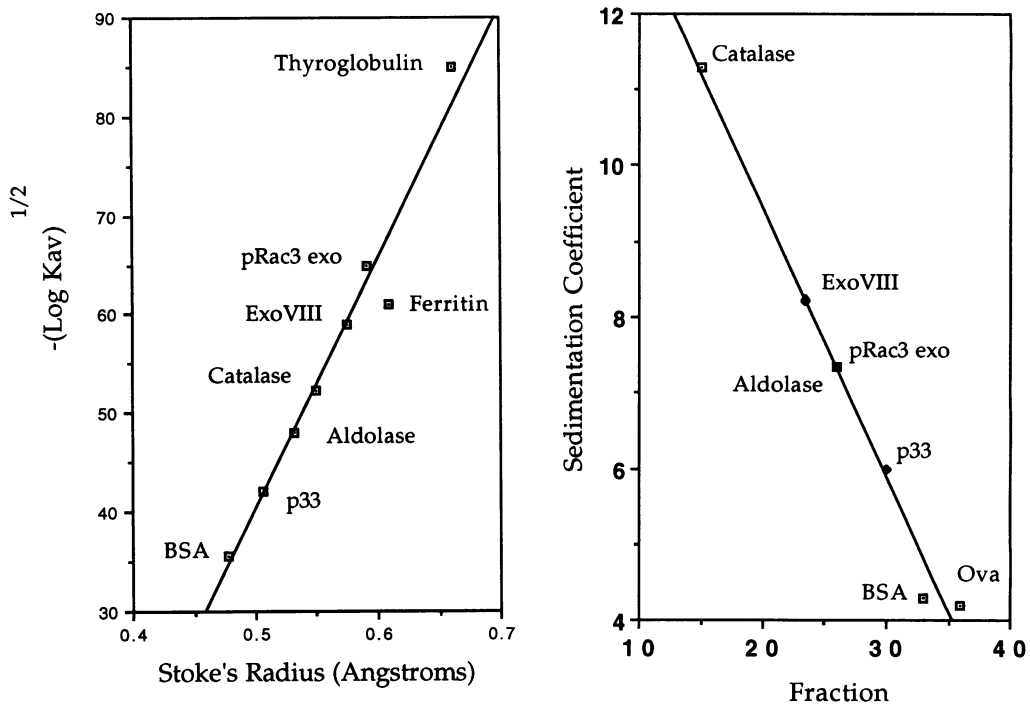

FIG. 4. Determination of the sedimentation coefficients and Stoke's radii for pRac3 exo and p33. The sedimentation coefficients and Stoke's radii of p33 and pRac3 exo were determined relative to those of protein standards as described in Materials and Methods. Proteins were detected by an enzymatic assay or by a Bradford protein assay. Standards: thyroglobulin (85 Å), ferritin (17.5S, 61 Å), exoVIII (8.45S, 59 Å [value redetermined; unpublished results]), catalase (11.3S, 52.2 Å), aldolase (7.35S, 48.1 Å), bovine serum albumin (4.3S, 35.5 Å) (BSA), and ovalbumin (4.2S, 30.5 Å) (Ova).

the addition of 200 mM KCl, whereas the addition of 500 mM KCl markedly decreased complex formation. The formation of p33–single-stranded DNA complexes was also insensitive to pH in the range of 6.0 to 8.5 (see Fig. 8).

Renaturation of homologous single-stranded DNA by p33. The ability of bacteriophage λ β protein to promote the renaturation of homologous single-stranded DNA suggested that p33 may promote a similar reaction (21, 39). The renaturation of homologous single-stranded DNA was initially detected by use of agarose gel electrophoresis to measure the ability of p33 to promote the conversion of denatured bacteriophage T7 DNA to high-molecular-weight aggregates that could not enter a gel (Fig. 6A), like that observed with RecA protein (38) and other proteins that promote the renaturation of DNA, such as *Saccharomyces cerevisiae* SEP1 and SF1 (16, 40). Such aggregates form when renaturation is initiated by nucleation between different sites on multiple homologous DNA molecules. The formation of these products was dependent on incubation with p33, and these products stained intensely with ethidium bromide, a result suggesting that they contained significant amounts of double-stranded DNA. The reaction did not require ATP or an ATP-regenerating system (data not shown). Similar results were observed when denatured linear M13mp19 RF DNA was used as a substrate (data not shown). Control experiments (Fig. 6B) indicated that the products were not protein-DNA complexes that were insensitive to treatment with proteinase K and $NaDodSO_4$. M13mp19 viral (single-stranded) DNA was incubated with either SSB or p33 and then processed without or with subsequent treatment with $NaDodSO_4$ and proteinase K. The binding of SSB converted the viral DNA to a form that was not visible by staining with ethidium bromide, presumably because it lacked the secondary structure required for the intercalation of ethidium bromide (Fig. 6B, lane 2). Treatment with $NaDodSO_4$ and proteinase K converted the complexes to free viral DNA (Fig. 6B, lane 1). Likewise, the binding of p33 to viral DNA produced complexes that either did not enter the gel or migrated as a broad smear (Fig. 6B, lane 4), and treatment with $NaDodSO_4$ and proteinase K converted these complexes to free viral DNA (lane 3).

To further characterize the renaturation reaction, we measured the ability of p33 to promote the conversion of S1 nuclease-sensitive homologous single-stranded DNA to S1 nuclease-resistant double-stranded DNA products (Fig. 7). The results demonstrated that p33 converted homologous single-stranded T7 DNA (heat-denatured linear double-stranded DNA) to S1 nuclease-resistant products but did not convert single-stranded M13mp19 DNA (single-stranded viral plus-strand DNA) to S1 nuclease-resistant products (Fig. 7A). The control experiment with M13mp19 viral DNA indicated that the formation of S1 nuclease-resistant products was not due to protection of the DNA by p33. The renaturation reaction was rapid and was complete within the

TABLE 2. Hydrodynamic properties of pRac3 exo and p33

Protein	Subunit mol wt	Stoke's radius (Å)	Native mol wt[a]	Sedimentation coefficient	Frictional coefficient (f/f_o)
pRac3 exo	42,500	65	253,600	7.3	1.23
p33	33,100	42	139,000	6.0	1.56

[a] Assuming a partial specific volume of 0.72 $cm^3\ g^{-1}$.

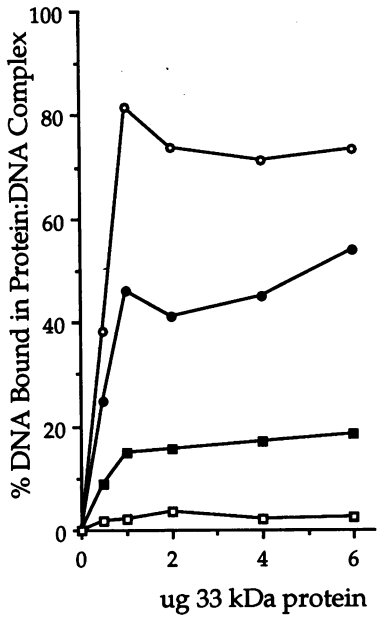

FIG. 5. Filter binding analysis of the DNA binding activity of p33. The DNA binding activity of p33 was determined by quantitating the retention of protein-DNA complexes formed between ^{3}H-single-stranded M13mp19 viral DNA or ^{3}H-linear double-stranded M13mp19 RF DNA and p33 on nitrocellulose filters as described in Materials and Methods. Each reaction mixture contained the indicated amount of protein and was incubated for 10 min at 37°C. Symbols: ○, single-stranded M13mp19 viral DNA with no KCL added; ●, single-stranded M13mp19 viral DNA with 200 mM KCl added; ■, single-stranded M13mp19 viral DNA with 500 mM KCl added; □, double-stranded linear M13mp19 RF DNA with no KCl added.

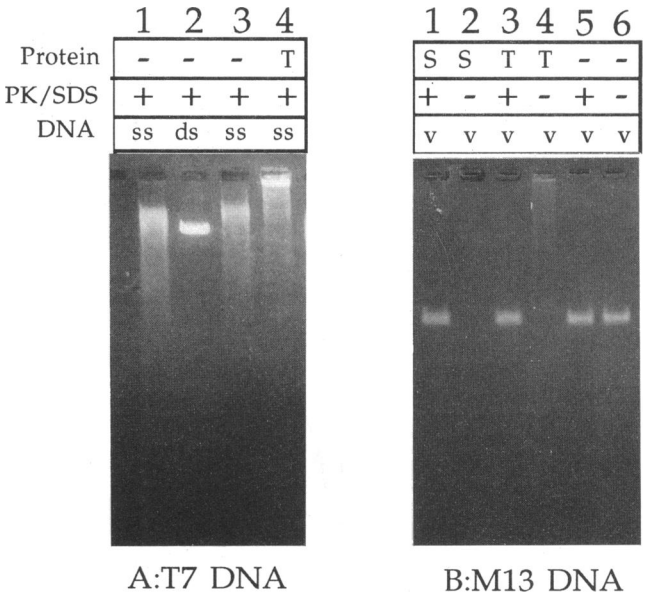

FIG. 6. Electrophoretic analysis of renaturation promoted by p33. (A) Renaturation of denatured bacteriophage T7 DNA was detected by agarose gel electrophoresis as described in Materials and Methods. Lanes: 1, heat-denatured T7 DNA incubated at 0°C; 2, native T7 DNA incubated at 0°C; 3, heat-denatured T7 DNA incubated at 37°C; 4, heat-denatured T7 DNA incubated at 37°C in the presence of 3 μg of p33 (T). (B) Control reaction mixtures (30 μl) containing 400 ng of M13mp19 viral DNA were incubated at 37°C with SSB (S) (3.8 μg) or p33 (3 μg) (T) and then treated or not treated with $NaDodSO_4$ (SDS) and proteinase K (PK) as described in Materials and Methods. Lanes: 1, incubation with p33 followed by incubation with $NaDodSO_4$ and proteinase K; 2, incubation with p33 followed by incubation without $NaDodSO_4$ and proteinase K; 3, incubation with SSB followed by incubation with $NaDodSO_4$ and proteinase K; 4, incubation with SSB followed by incubation without $NaDodSO_4$ and proteinase K; 5, incubation without protein followed by incubation with $NaDodSO_4$ and proteinase K; 6, incubation without protein followed by incubation without $NaDodSO_4$ and proteinase K. ss, single stranded; ds, double stranded; v, viral.

first 10 min (Fig. 7B). The reaction requirements for renaturation are summarized in Table 4 and differ from the requirements for binding to single-stranded DNA. The renaturation reaction had a significant requirement for $MgCl_2$ and also appeared to require at least one reduced sulfhydryl group. The addition of 100 mM KCl increased the reaction rate by 44% (Table 4), and higher concentrations of KCl were inhibitory (data not shown). Potassium glutamate at 100 mM had no effect on the reaction, and 100 mM NaCl stimulated the reaction slightly (Table 4). The effect of different pHs on renaturation was tested (Fig. 8), and the rate of renaturation was found to be relatively insensitive to changes in pH between 7 and 8.5.

TABLE 3. Reaction requirements for binding of p33 to single-stranded DNA

Reaction mixture[a]	% Relative binding[b]
Complete	100
− $MgCl_2$	91.7
− DTT	95.8
− DTT, + 5 mM NEM	75.0
+ 200 mM KCl	71.7
+ 500 mM KCl	13.9

[a] Each complete reaction mixture (30 μl) contained 33 mM Tris (pH 7.5), 13 mM $MgCl_2$, 1.8 mM dithiothreitol (DTT), 88 μg of bovine serum albumin per ml, 400 ng of ^{3}H-single-stranded M13mp19 viral DNA, and 1 μg of p33. Reaction mixtures were incubated at 37°C and processed as described in the legend to Fig. 5 and in Materials and Methods. Additions (+) and omissions (−) of reaction components were as indicated. NEM, *N*-ethylmaleimide.

[b] One hundred percent relative binding was equal to 63.5% of the ^{3}H-DNA bound.

DISCUSSION

Genetic and biochemical analyses have demonstrated that the *E. coli recE* region encodes a 140,000-molecular-weight exonuclease, called exoVIII, that is required for recombination in *recB recC sbcA* mutants (4, 9, 13, 19, 20, 22, 31, 32, 49, 54). This exonuclease can be truncated at its amino terminus and still retain enzymatic and biological activities (4, 8, 31, 55). Clark and colleagues (9) have demonstrated that the *recE* region contains two overlapping reading frames, now called *recE* and *recT*, capable of encoding 96.2- and 29.5-kDa proteins, respectively. They suggest that the synthesis of full-length exoVIII is likely to involve a translational frameshift event that fuses the products of the *recE* and *recT* ORFs, a process resulting in a 125.9-kDa protein; this size is consistent with the size of exoVIII as determined by $NaDodSO_4$-PAGE. In this paper, we have demonstrated that plasmids that direct the expression of either exoVIII or a truncated derivative, pRac3 exo, coexpress a 33,000-

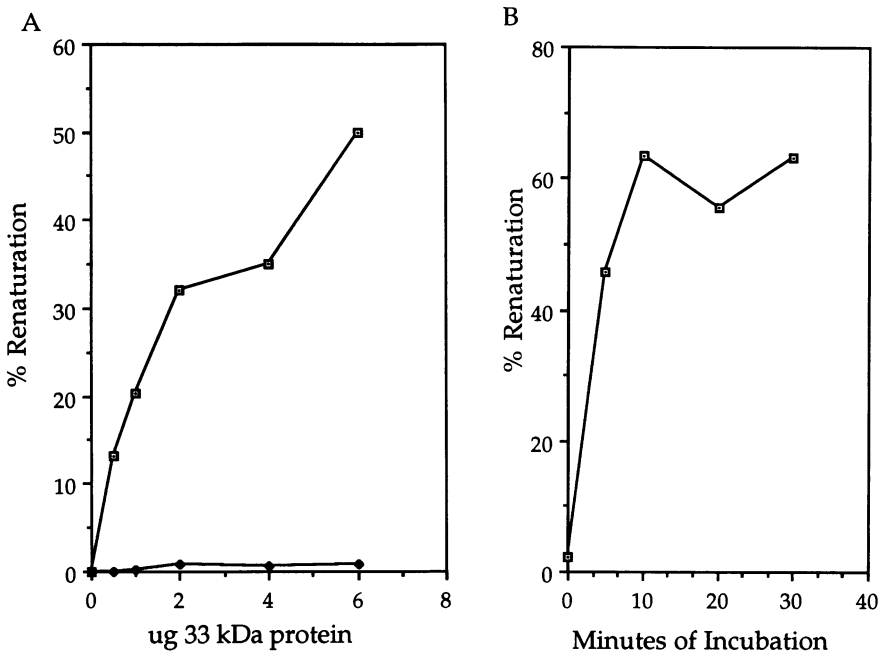

FIG. 7. S1 nuclease assay of p33-promoted renaturation of homologous single-stranded DNA. (A) The formation of double-stranded DNA from heat-denatured ^{3}H-T7 DNA (⊡) or from M13mp19 viral DNA (◆) promoted by the indicated amounts of p33 was determined by the S1 nuclease assay as described in Materials and Methods. Each reaction mixture was incubated for 10 min at 37°C. (B) The time course of the formation of double-stranded DNA from heat-denatured ^{3}H-T7 DNA promoted by p33 was determined by the S1 nuclease assay as described in Materials and Methods. Each reaction mixture was incubated for the indicated period of time at 37°C and contained 3 μg of p33.

molecular-weight protein. p33 is coexpressed with pRac3 exo and exoVIII at molar ratios of 3:1 and 10:1, respectively. This protein has been purified to homogeneity and analyzed. N-terminal sequence analysis has shown that the N-terminal Met of p33 is encoded by the first Met codon of the *recT* ORF and that the size of p33 is consistent with the size predicted for the product of the *recT* ORF (9). In addition, there is a ribosome binding site at an optimal distance upstream of this first *recT* Met codon. These observations indicate that p33 is the product of the *recT* gene. Biochemical analysis of the RecT protein has demonstrated that it binds specifically to single-stranded DNA and promotes the renaturation of complementary single-stranded DNA.

Genetic analysis of both plasmid recombination and recombination of λ *red gam* phage has implicated additional activities besides the exonuclease activity of exoVIII that are required for RecE pathway recombination. Recombination of both circular plasmid substrates and linear dimer plasmid substrates and recombination of λ *red gam* phage in *recB recC sbcA E. coli* strains do not require *recA* (10, 11, 13, 18, 23, 32, 49). Assuming that some type of homologous pairing function is required for these types of recombination, it is likely that the *recE* region encodes such an activity. Expression of the *recE* region substitutes for the two different recombination genes, *redα* and *redβ*, encoded by bacteriophage λ (11, 13, 14). Because exoVIII is biochemically similar to the λ *redα* gene product λ exo, it is likely that the *recE* region encodes a second activity that can substitute for that of the *redβ* gene product λ β protein. Genetic evidence that λ β protein in *recB recC* mutants substitutes for RecA (2) combined with the biochemical properties of λ β protein suggests that the *recE* region may encode some type of homologous pairing protein that is a λ β protein analog. The results presented here demonstrate that RecT protein binds to single-stranded DNA and promotes the renaturation of complementary single-stranded DNA and is therefore biochemically similar to λ β protein. We suggest that RecT protein is an analog of λ β protein and is the homologous pairing protein that is expressed in *recB recC sbcA* mutants and that substitutes for RecA.

β protein was initially identified as an antigenically distinct factor that was copurified with λ exo (44). β protein and RecT protein are biochemically similar in that they bind single-stranded DNA and promote the renaturation of complementary single-stranded DNA (21, 39). However, the two proteins vary slightly in size (molecular weights of 33,000 for RecT and 28,000 for β protein) and do not appear to share any significant amino acid sequence homology (9, 15). The

TABLE 4. Reaction requirements for renaturation of complementary single-stranded DNA by p33

Reaction mixture[a]	% Relative renaturation[b]
Complete	100
− $MgCl_2$	25.3
− DTT	38.6
− DTT, + 5 mM NEM	23.3
− KCl	55.8
− KCl, + 100 mM KGlu	45.1
− KCl, + 100 mM NaCl	84.3

[a] Each complete reaction mixture (30 μl) contained 33 mM Tris (pH 7.5), 13 mM $MgCl_2$, 1.8 mM dithiothreitol (DTT), 100 mM KCl, 3 μg of p33, and 500 ng of heat-denatured double-stranded ^{3}H-T7 DNA. The reactions were processed for the S1 nuclease assay as described in the legend to Fig. 7 and in Materials and Methods. Additions (+) and omissions (−) are as indicated. NEM, *N*-ethylmaleimide; KGlu, potassium glutamate.

[b] One hundred percent renaturation was equal to 45.9% of input DNA converted to S1 nuclease-resistant product.

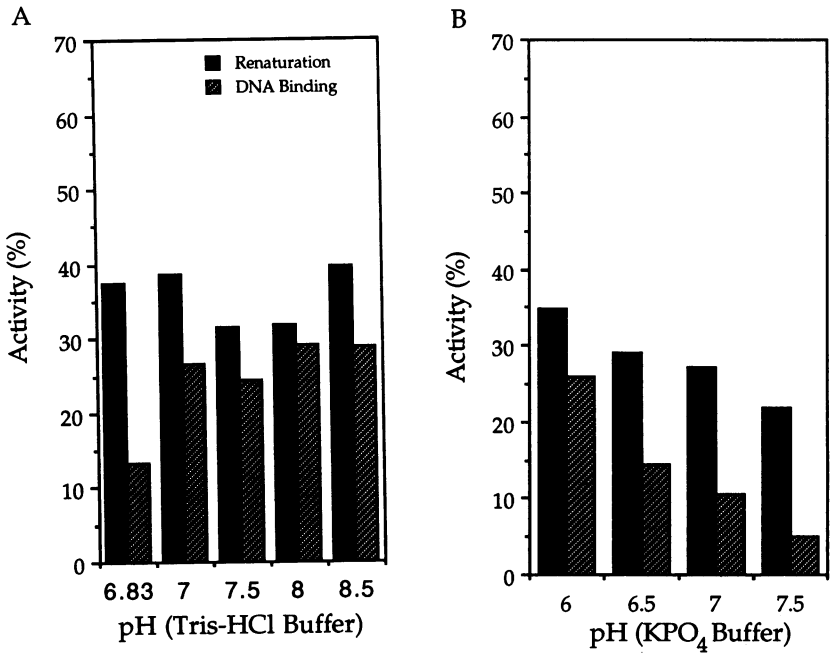

FIG. 8. pH dependence of binding to single-stranded DNA and DNA renaturation promoted by p33. Binding to single-stranded DNA and renaturation of denatured DNA were measured as described in Materials and Methods, except that 33 mM Tris at pH 6.83, 7.0, 8.0, or 8.5 (A) or 33 mM KPO_4 at pH 6.0, 6.5, 7.0, or 7.5 (B) was substituted for the 33 mM Tris (pH 7.5) that was present in the standard reaction mixture. pH determinations of stock buffers were made at 25°C at buffer concentrations of 50 mM.

abilities of these two proteins to promote the renaturation of homologous single-stranded DNA also appear to be qualitatively different. β protein has been shown to promote renaturation only in a narrow pH range centered around pH 6.0 (21, 39), whereas RecT protein efficiently promotes renaturation at pHs at least as high as 8.5. β protein also stimulates the RecA-mediated formation of D-loops and facilitates the RecA-mediated pairing and subsequent strand transfer of linear duplex DNA and single-stranded circular DNA substrates (39). The ability of RecT protein to stimulate RecA protein during an in vitro strand transfer reaction has not yet been tested. A more extensive analysis of both proteins is required to obtain a complete picture of their biochemical differences and similarities and to determine whether they have other activities related to homologous pairing and recombination. However, the two proteins appear to be biochemically similar enough to be consistent with the observation that RecT protein can substitute for β protein in recombination.

The structures of the *recE* and *recT* ORFs indicate that while RecT protein is expressed independently of exoVIII (this work), the C-terminal 33,000-molecular-weight portion of exoVIII must be identical to RecT protein (9). At present, it is unclear what role this C-terminal portion of exoVIII plays and what role the independent overexpression of RecT plays. A genetic analysis done by Clark et al. has indicated that *recE* and *recT* have different functions (9). Moreover, the RecT portion of exoVIII is unlikely to be required for the exonuclease activity of exoVIII (4, 9, 31). A number of possibilities that could explain these results exist. (i) The RecT portion of exoVIII could be inactive, leading to a requirement for the independent expression of RecT. (ii) The RecT portion of exoVIII could have homologous pairing activity, but the level of exoVIII produced could be insufficient to promote recombination. (iii) Although identical in amino acid sequence, the RecT portion of exoVIII and free RecT protein could have different functions. For example, the covalently linked RecT portion of exoVIII could act as a homologous pairing protein and the free RecT protein could act as an accessory factor or function in a different step, such as the promotion of branch migration. Further analysis will be required to determine the significance of the two forms of RecT protein.

The observations that β protein can substitute for RecA protein (2) under some circumstances and that RecT protein is similar to β protein provide an explanation for why some recombination events in *recB recC sbcA* mutants do not require RecA. At present, there is no evidence that either β protein or RecT protein promotes the types of strand exchange reactions that RecA protein promotes. This observation is consistent with the observation that the products of the *recE* coding region (namely, exoVIII and RecT protein) cannot substitute for RecA protein in either conjugational recombination or P1 transduction (11, 13, 32) and underscores the mechanistic difference(s) that must exist between this type of recombination and plasmid recombination or recombination of λ *red* phage. It is clear that one of the major challenges in understanding the mechanism(s) of recombination in *E. coli* is relating the biochemical properties of the different homologous pairing proteins to the different recombination mechanisms that exist.

ACKNOWLEDGMENTS

We thank Cindy Luisi-DeLuca, David Norris, Nai-Wen Chi, A. J. Clark, W.-D. Heyer, Arlen Johnson, and Eric Alani for helpful discussions, communication of unpublished data, technical advice, and comments on the manuscript.

This work was supported by National Institutes of Health grant GM26017 to R.D.K.

REFERENCES

1. **Barbour, S. D., H. Nagaishi, A. Templin, and A. J. Clark.** 1970. Biochemical and genetic studies of recombination proficiency in *Escherichia coli*. II. Rec$^+$ revertants caused by indirect suppression of rec$^-$ mutations. Proc. Natl. Acad. Sci. USA **67:**128–135.
2. **Berger, I., and A. Cohen.** 1989. Suppression of RecA deficiency in plasmid recombination by bacteriophage λ β protein in *recBCD exoI Escherichia coli* cells. J. Bacteriol. **171:**3523–3529.
3. **Chi, N., and R. D. Kolodner.** Unpublished results.
4. **Chu, C., A. Templin, and A. J. Clark.** 1989. Suppression of a frameshift mutation in the *recE* gene of *Escherichia coli* K-12 occurs by gene fusion. J. Bacteriol. **171:**2101–2109.
5. **Clark, A. J.** 1971. Toward a metabolic interpretation of genetic recombination of *E. coli* and its phages. Annu. Rev. Microbiol. **25:**438–464.
6. **Clark, A. J.** 1973. Recombination-deficient mutants of *E. coli* and other bacteria. Annu. Rev. Genet. **7:**67–86.
7. **Clark, A. J.** 1974. Progress toward a metabolic interpretation of genetic recombination of *Escherichia coli* and bacteriophage λ. Genetics **78:**259–271.
8. **Clark, A. J., S. J. Sandler, D. K. Willis, C. C. Chu, M. A. Blanar, and S. T. Lovett.** 1984. Genes of the RecE and RecF pathways of conjugational recombination in *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. **49:**453–462.
9. **Clark, A. J., V. Sharma, S. Brenowitz, C. C. Chu, L. Satin, A. Templin, I. Berger, and A. Cohen.** Genetic and molecular analysis of the C-terminal region of the *recE* gene from the rac prophage by *Escherichia coli* K-12. Submitted for publication.
10. **Fishel, R. A., A. J. James, and R. D. Kolodner.** 1981. *recA*-independent general genetic recombination of plasmids. Nature (London) **294:**184–186.
11. **Gillen, J. R., and A. J. Clark.** 1974. The RecE pathway of bacterial recombination, p. 123–136. *In* R. F. Grell (ed.), Mechanisms in recombination. Plenum Press, New York.
12. **Gillen, J. R., A. E. Karu, H. Nagaishi, and A. J. Clark.** 1977. Characterization of the deoxyribonuclease determined by lambda reverse as exonuclease VIII of *Escherichia coli*. J. Mol. Biol. **113:**27–41.
13. **Gillen, J. R., D. K. Willis, and A. J. Clark.** 1981. Genetic analysis of the RecE pathway of genetic recombination in *Escherichia coli* K-12. J. Bacteriol. **145:**521–535.
14. **Gottesman, M. M., M. E. Gottesman, S. Gottesman, and M. Gellert.** 1974. Characterization of bacteriophage λ reverse as an *Escherichia coli* phage carrying a unique set of host-derived recombination functions. J. Mol. Biol. **88:**471–487.
15. **Hall, S. D., and R. D. Kolodner.** Unpublished results.
16. **Heyer, W., and R. D. Kolodner.** 1988. Renaturation of DNA by a *Saccharomyces cerevisiae* protein that catalyzes homologous pairing and strand exchange. J. Biol. Chem. **263:**15189–15195.
17. **Horii, Z., and A. J. Clark.** 1973. Genetic analysis of the RecF pathway to genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. J. Mol. Biol. **80:**327–344.
18. **James, A. A., P. T. Morrison, and R. D. Kolodner.** 1982. Genetic recombination of bacterial plasmid DNA: analysis of the effect of recombination-deficient mutations on plasmid recombination. J. Mol. Biol. **160:**411–430.
19. **Joseph, J. W., and R. D. Kolodner.** 1983. Exonuclease VIII of *Escherichia coli*. I. Purification and physical properties. J. Biol. Chem. **258:**10411–10417.
20. **Joseph, J. W., and R. D. Kolodner.** 1983. Exonuclease VIII of *Escherichia coli*. II. Mechanism of action. J. Biol. Chem. **258:**10418–10424.
21. **Kmiec, E., and W. K. Holloman.** 1981. β protein of bacteriophage λ promotes renaturation of DNA. J. Biol. Chem. **256:**12636–12639.
22. **Kushner, S. R., H. Nagaishi, and A. J. Clark.** 1974. Isolation of exonuclease VIII: the enzyme associated with the *sbcA* indirect suppressor. Proc. Natl. Acad. Sci. USA **71:**3593–3597.
23. **Laban, A., and A. Cohen.** 1981. Interplasmidic and intraplasmidic recombination in *Escherichia coli* K-12. Mol. Gen. Genet. **184:**200–207.
24. **Little, J. W.** 1967. An exonuclease induced by bacteriophage λ. II. Nature of the enzymatic reaction. J. Biol. Chem. **242:**679–686.
25. **Little, J. W., I. R. Lehman, and A. D. Kaiser.** 1967. An exonuclease induced by bacteriophage λ. I. Preparation of the crystalline enzyme. J. Biol. Chem. **242:**672–678.
26. **Lloyd, R. G., and C. Buckman.** 1985. Identification and genetic analysis of *sbcC* mutations in commonly used *recBC sbcB* strains of *Escherichia coli* K-12. J. Bacteriol. **164:**836–844.
27. **Lloyd, R. G., C. Buckman, and F. E. Benson.** 1987. Genetic analysis of conjugational recombination in *Escherichia coli* K12 strains deficient in RecBCD enzyme. J. Gen. Microbiol. **133:**2531–2538.
28. **Lloyd, R. G., M. C. Porton, and C. Buckman.** 1988. Effect of *recF*, *recJ*, *recN*, *recO* and *ruv* mutations on ultraviolet survival and genetic recombination in a *recD* strain of *Escherichia coli* K12. Mol. Gen. Genet. **212:**317–324.
29. **Lovett, S. T., and A. J. Clark.** 1984. Genetic analysis of the *recJ* gene of *Escherichia coli* K-12. J. Bacteriol. **157:**190–196.
30. **Lovett, S. T., C. Luisi-DeLuca, and R. D. Kolodner.** 1988. The genetic dependence of recombination in *recD* mutants of *Escherichia coli*. Genetics **120:**37–45.
31. **Luisi-DeLuca, C., A. J. Clark, and R. D. Kolodner.** 1988. Analysis of the *recE* locus of *Escherichia coli* K-12 by use of polyclonal antibodies to exonuclease VIII. J. Bacteriol. **170:**5797–5805.
32. **Luisi-DeLuca, C., S. T. Lovett, and R. D. Kolodner.** 1989. Genetic and physical analysis of plasmid recombination in *recB recC sbcB* and *recB recC sbcA Escherichia coli* K-12 mutants. Genetics **122:**269–278.
33. **Mahajan, S. K.** 1988. Pathways of homologous recombination in *Escherichia coli*, p. 87–140. *In* R. Kucherlapati and G. R. Smith (ed.), Genetic recombination. American Society for Microbiology, Washington, D.C.
34. **Mahajan, S. K., and A. R. Datta.** 1979. Mechanism of recombination by the RecBC and the RecF pathways following conjugation in *Escherichia coli* K12. Mol. Gen. Genet. **169:**67–78.
35. **Mahdi, A. A., and R. G. Lloyd.** 1989. Identification of the *recR* locus of *Escherichia coli* K-12 and analysis of its role in recombination and DNA repair. Mol. Gen. Genet. **216:**503–510.
36. **Manservigi, R., P. Spear, and A. Buchan.** 1977. Cell fusion induced by herpes simplex virus is promoted and suppressed by different viral glycoproteins. Proc. Natl. Acad. Sci. USA **74:**3913–3917.
37. **Matsudaira, P.** 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. J. Biol. Chem. **262:**10035–10038.
38. **McEntee, K., G. M. Weinstock, and I. R. Lehman.** 1980. RecA protein-catalyzed strand assimilation: stimulation by *Escherichia coli* single-stranded DNA-binding protein. Proc. Natl. Acad. Sci. USA **77:**857–861.
39. **Muniyappa, K., and C. M. Radding.** 1986. The homologous recombination system of phage λ. J. Biol. Chem. **261:**7472–7478.
40. **Norris, D., and R. D. Kolodner.** 1990. Interaction of a *Saccharomyces cerevisiae* strand exchange stimulatory factor with DNA. Biochemistry **29:**7911–7917.
41. **Radding, C. M.** 1970. The role of exonuclease and β protein of bacteriophage λ in genetic recombination. I. Effects of *red* mutants on protein structure. J. Mol. Biol. **52:**491–499.
42. **Radding, C. M., and D. M. Carter.** 1971. The role of exonuclease and β protein of phage λ in genetic recombination. J. Biol. Chem. **246:**2513–2518.
43. **Radding, C. M., J. Rosenzwieg, F. Richards, and E. Cassuto.** 1971. Appendix: separation and characterization of exonuclease, β protein, and a complex of both. J. Biol. Chem. **246:**2510–2512.
44. **Radding, C. M., and D. C. Shreffler.** 1966. Regulation of λ exonuclease. II. Joint regulation of exonuclease and a new λ antigen. J. Mol. Biol. **18:**251–261.
45. **Richardson, C. C.** 1966. The 5′-terminal nucleotides of T7 bacteriophage deoxyribonucleic acid. J. Mol. Biol. **15:**49–61.

46. **Sawitzke, J. A., and F. W. Stahl.** 1992. Phage λ has an analog of *Escherichia coli recO*, *recR* and *recF* genes. Genetics **130:**7–16.
47. **Siegel, L. M., and K. J. Monty.** 1966. Determination of molecular weights and frictional ratios of proteins in impure systems by use of gel filtration and density gradient centrifugation. Biochim. Biophys. Acta **112:**346–362.
48. **Smith, G. R.** 1988. Homologous recombination in prokaryotes. Microbiol. Rev. **52:**1–28.
49. **Symington, L. S., P. T. Morrison, and R. D. Kolodner.** 1985. Intramolecular recombination of linear DNA catalyzed by the *Escherichia coli* RecE recombination system. J. Mol. Biol. **186:**515–525.
50. **Templin, A., S. R. Kushner, and A. J. Clark.** 1972. Genetic analysis of mutations indirectly suppressing *recB* and *recC* mutations. Genetics **72:**205–215.
51. **Unger, R. C., H. Echols, and A. J. Clark.** 1972. Interaction of the recombination pathways of bacteriophage λ and host *Escherichia coli*: effects on λ recombination. J. Mol. Biol. **70:**531–537.
52. **Wensink, P. C., D. J. Finnegan, P. J. Donelson, and D. S. Hogness.** 1974. A system for mapping DNA sequences in the chromosomes of *Drosophila melanogaster*. Cell **3:**315–325.
53. **Willetts, N. S., A. J. Clark, and B. Low.** 1969. Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. J. Bacteriol. **97:**244–249.
54. **Willis, D. K., K. E. Fouts, S. D. Barbour, and A. J. Clark.** 1983. Restriction nuclease and enzymatic analysis of transposon-induced mutations of the Rac prophage which affect expression and function of *recE* in *Escherichia coli* K-12. J. Bacteriol. **156:**727–736.
55. **Willis, D. K., L. H. Satin, and A. J. Clark.** 1985. Mutation-dependent suppression of *recB21 recC22* by a region cloned from the Rac prophage of *Escherichia coli* K-12. J. Bacteriol. **162:**1166–1172.