JOURNAL OF BACTERIOLOGY, Jan. 1984, p. 21–27
0021-9193/84/010021-07$02.00/0

Vol. 157, No. 1

Physical and Biochemical Analysis of the Cloned *recB* and *recC* Genes of *Escherichia coli* K-12

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Received 30 August 1983/Accepted 15 October 1983

A 19-kilobase *Bam*HI fragment encoding the *recB* (exonuclease V), *recC* (exonuclease V), *ptr* (protease III), *thyA*, and *argA* genes of *Escherichia coli* K-12 was cloned into a multicopy plasmid (pCDK3). In *E. coli* maxicells, the plasmid specified the synthesis of seven polypeptides of 140,000 (*recC*), 128,000 (*recB*), 110,000 (*ptr*), 53,000 (*argA*), 50,000, 33,000 (*thyA*), and 22,000 M_r, as well as β-lactamase and chloramphenicol acetyltransferase. From analysis of subclones and Tn*1000* insertions, it appears that the 110,000- and 50,000-M_r proteins originated from the *ptr* DNA coding sequence which is located between the *recB* and *recC* genes. Although *recC*, *ptr*, and *recB* were physically closely linked and transcribed in the same direction, they do not appear to constitute an operon. Cells carrying pCDK3 contained a 30- to 50-fold increase in exonuclease V activity, without affecting cell viability.

recB and *recC* mutants of *Escherichia coli* K-12 are phenotypically recombination deficient, sensitive to DNA-damaging agents, and segregate a large fraction of inviable progeny (8). Both genes have been shown to encode subunits of exonuclease V (22, 23, 40), an enzyme which functions in vitro as an ATP-dependent exonuclease, an ATP-stimulated endonuclease, a DNA-dependent ATPase, and a DNA helicase (31, 32). The determination of the actual biochemical role of exonuclease V in the cell, however, has been hampered in part by the difficulty in obtaining large quantities of the enzyme and by the lack of temperature-sensitive mutations in the structural genes (22).

Amplification of exonuclease V would clearly facilitate the analysis of this multifunctional enzyme. Recently, Hickson and Emmerson (17) reported the isolation of the *recB* and *recC* genes on separate *Bam*HI fragments. Our paper describes the cloning of *recB* and *recC* on a single 19-kilobase (kb) *Bam*HI DNA fragment. Maxicell analysis of various Tn*1000* insertions in the recombinant plasmid pCDK3 has additionally demonstrated that the structural gene for protease III (*ptr* encodes a 110,000-M_r protein) maps between the *recB* and *recC* loci. All three genes are independently transcribed. The presence of pCDK3 in wild-type strains of *E. coli* results in a 30- to 50-fold increase in exonuclease V activity.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The relevant genotypes of the *E. coli* K-12 strains used in this work are listed in Table 1. Strain AC113, carrying a deletion from *thyA* through *argA*, was graciously supplied by A. Chaudhury and will be described in more detail elsewhere. Subclones and Tn*1000* insertions are indicated in Fig. 1. Media used were Luria broth and broth solidified with 2% agar (22), K medium (41), and NZY broth (4). Media were supplemented with the appropriate antibiotics and amino acids as needed.

Enzymes. T4 DNA ligase and *Cla*I were purified in this laboratory. *Pst*I was a gift from R. A. Lansman. R. B. Meagher provided *Bgl*II. All other restriction endonucleases were obtained from Bethesda Research Laboratories and were used according to the instructions of the manufacturer. RNase IIA, DNase I, and lysozyme were purchased from Sigma Chemical Co. DNA polymerase I was obtained from Boehringer Mannheim Co. Proteinase K was purchased from Beckman Co.

Materials. Materials were obtained from the following sources: agarose, Sea Kem Co.; *N,N,N',N'*-tetramethylethylenediamine and ammonium persulfate, Bio-Rad Laboratories; tetracycline, chloramphenicol, ampicillin, kanamycin, cycloserine, and dithiothreitol. Sigma; Sephadex G-100 and protein molecular weight standards, Pharmacia Fine Chemicals; deoxynucleoside 5'-triphosphates, P-L Biochemicals, Inc.; DEAE-cellulose, Whatman, Inc.; $(NH_4)_2SO_4$, insulin chain B, and enzyme grade sucrose, Schwarz/Mann; cesium chloride, Penn Rare Metals Co.; [^{3}H]thymidine, New England Nuclear Corp.; [α-^{32}P]dATP (400 Ci/mmol) and [^{35}S]methionine (400 Ci/mmol), Amersham Corp. All other chemicals were of analytical grade.

DNA isolation. *E. coli* chromosomal DNA was isolated by the method of Davis and Vapnek (10). Large-scale plasmid DNA isolation was by the method of Birnboim and Doly (3). Rapid screens of plasmid DNAs were performed by the method of Ish-Horowicz and Burke (19). Radioactively labeled plasmid DNA was prepared by the method of Rigby et al. (35). Bacteriophage lambda DNA was isolated by the method of Blattner et al. (4).

Genetic techniques. Mutations in *recB* or *recC* or both cause strains to become sensitive to UV light (13). Complementation of *recB* and *recC* chromosomal mutations by recombinant plasmids was detected by using the replica-plating test for UV resistance developed by Clark and Margulies (9). Quantitative UV survival tests were performed as described previously by Kushner (22).

The presence of the *thyA*$^+$ and *argA*$^+$ genes was tested by the ability of strains to grow on minimal medium lacking thymine or arginine, respectively.

Plasmid DNAs were transformed into various genetic backgrounds by the method of Kushner (24).

Construction of pDP3. A 6.4-kb *Kpn*I fragment containing the gene for kanamycin resistance was isolated from plasmid pML21 (20). This DNA fragment was ligated to a 3.2-kb *Kpn*I fragment containing the F replication origin of pDF41 (20) and used to transform strain SK1592. Transformants

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TABLE 1. Bacterial strains used

Strain	Characteristics[a]						Source or derivation
	argA	*recB*	*recC*	*thyA*	Other markers	Plasmid	
AB1157	+	+	+	+			18
AC113	Δ	Δ	Δ	Δ			G. Smith
BW1001	+	+	+	+	*xonA101*		B. Weiss
C600	+	+	+	+			D. Vapnek
CSR603	+	+	+	+	*recA1 uvrA6 phr-1*		36
JC4456	+	+	*73*	+			44
JC4457	+	*58*	+	+			44
JC4535	+	*60*	+	+			44
JC5422	+	+	+	*325*			44
JC5489	+	+	*22*	+			44
JC5519	+	*21*	*22*	+			44
JC5703	+	+	*82*	+			44
JC5743	+	*21*	+	+			44
KL399	+	+	+	+	*recA200*		27
MV10	+	+	+	+	Δ*trpE*		20
NK5992	Tn*10*	+	+	+			N. Kleckner
Q359	+	+	+	+	*hsdR*		21
SK679		*95*	+	+			S. R. Kushner
SK4503	Tn*10*	+	+	+	Δ*trpE*		MV10 × NK5992 Tc^r transductant
SK4526	Tn*10*	+	+	+	Δ*trpE*	pCDK1 (*argA*$^+$)	SK4503 transformant
SK4538	Tn*10*	+	+	+	Δ*trpE*	pCDK2 (*argA*$^+$)	SK4503 transformant
SK4544	+	+	+	+		pBR325	AB1157 transformant
SK4545	+	+	+	+		pCDK3 (*argA*$^+$ *recB*$^+$ *recC*$^+$ *thyA*$^+$)	AB1157 transformant
SK4548	+	*21*	+	+		pBR325	JC5743 transformant
SK4549	+	*21*	+	+		pCDK3	JC5743 transformant
SK4550	+	+	*22*	+		pBR325	JC5489 transformant
SK4551	+	+	*22*	+		pCDK3	JC5489 transformant
SK4552	+	*21*	*22*	+		pBR325	JC5519 transformant
SK4553	+	*21*	*22*	+		pCDK3	JC5519 transformant
SK5107	Δ	Δ	Δ	Δ		pCDK3::Tn*1000*-24	AC113 transformant
SK5108	Δ	Δ	Δ	Δ		pCDK3	AC113 transformant

[a] Δ indicates that all or part of the gene is deleted.

were selected on Luria agar plates containing 10 μg of kanamycin per ml. pDP3-containing strains of *E. coli* were resistant to up to 100 μg of kanamycin per ml.

Cloning of *argA*. A DNA fragment carrying *argA* was isolated by digesting pDP3 plasmid DNA and strain AB1157 chromosomal DNA with *Sal*I (which cuts pDP3 once), ligating in a 1:10 molar ratio of vector-chromosomal DNA with T4 DNA ligase, transforming into SK4503 (*argA*::Tn*10*), and selecting for Kan^r Tc^r (pCDK1) colonies, which grew in the absence of arginine. Plasmid DNA isolated from one such colony contained a 3-kb insert. This *Sal*I fragment was subsequently subcloned into the single *Sal*I site of pBR322 (pCDK2) and was shown to complement a variety of *argA* mutations.

Construction of the *E. coli* genomic library. Chromosomal DNA from *E. coli* BW1001 was partially digested with *Bam*HI and added to *Bam*HI-digested λ1059 DNA (21). The ratio of λ vector DNA to *E. coli* DNA in the ligation mixture was 2:1 at a total DNA concentration of 210 μg/ml. The ligation mixture was incubated overnight at 16°C and then packaged in vitro as described previously by Nagao et al. (33). A single amplification of each packaging reaction was carried out on strain Q359 (21) at nonconfluent phage densities (25) before the genomic library was screened.

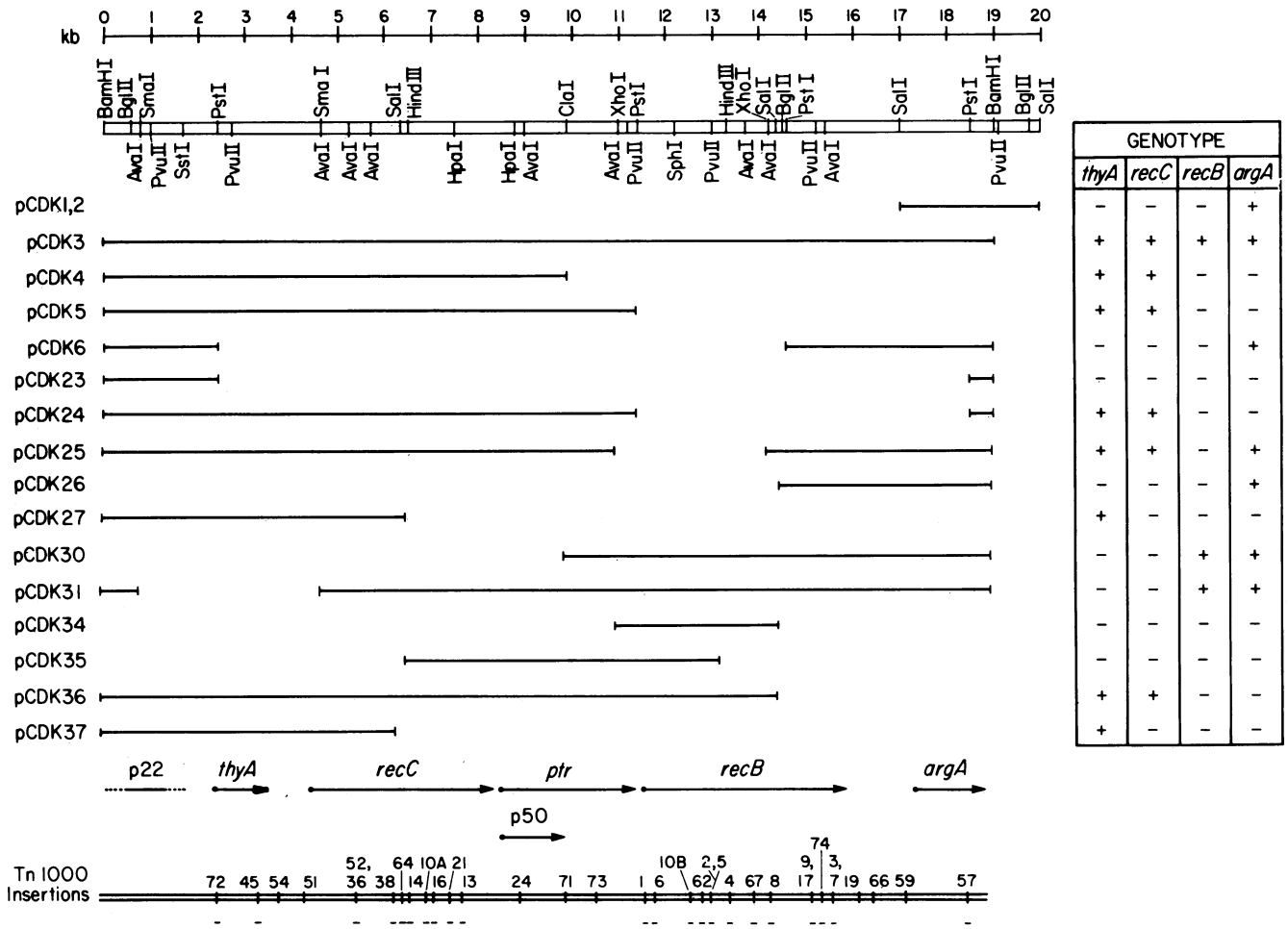
Isolation of *recB* and *recC* genes from the *E. coli* genomic library. The 3-kb *Sal*I fragment in pCDK2 contained a single *Bam*HI restriction site (data not shown). This plasmid was ^{32}P labeled by nick translation and used to probe the *E. coli* genomic library of *Bam*HI inserts in λ1059 by the method of Maniatis et al. (28).

Plaques that hybridized to pCDK2 were purified by two cycles of plating. Purified phage DNA was digested with various restriction enzymes, run on 0.8% agarose gels, transferred to nitrocellulose paper by the method of Southern (38), and subsequently probed with ^{32}P-labeled pCDK2 DNA. DNA fragments which hybridized were then subcloned into the single *Bam*HI site of pBR325 to permit testing for genetic complementation. Subclones were first isolated as Tc^s Cm^r transformants.

Transposon Tn*1000* insertions. Transposon Tn*1000* insertions into pCDK3 plasmid DNA were isolated by the method of Guyer (15). Inactivation of *thyA*, *argA*, *recB*, and *recC* was determined by genetic complementation. The physical location of each insertion was determined by restriction enzyme analysis.

Maxicells. Plasmid-encoded proteins were analyzed by the method of Sancar et al. (36). Plasmids were transformed into the maxicell strain CSR603, and plasmid-specific products were labeled with [^{35}S]methionine. Samples were electrophoresed in 7.5 or 10% sodium dodecyl sulfate-polyacrylamide gels (25) which were then dried in vacuo and autoradiographed.

Assay of exonuclease V activity and enzyme isolation. Cells were lysed by the method of Wickner et al. (42). Ammonium sulfate was added to 45% saturation with stirring to fractionate the crude extract. The resulting precipitate was centri-



thyA	recC	recB	argA
−	−	−	+
+	+	+	+
+	+	−	−
+	+	−	−
−	−	−	+
−	−	−	−
+	+	−	−
+	+	−	+
−	−	−	+
+	−	−	−
−	−	+	+
−	−	+	+
−	−	−	−
−	−	−	−
+	+	−	−
+	−	−	−

FIG. 1. Physical analysis of the *recBC* region of the *E. coli* chromosome and location of Tn*1000* insertions. Gene products and the direction of transcription are as indicated. The extent of the subclones used are as shown. Numbers toward the bottom of the map indicate the location of various Tn*1000* insertions, used in delineating gene locations and directions of transcription. Minus signs indicate loss of genetic complementation by a specific Tn*1000* insertion.

fuged at 10,000 × *g* for 10 min. and suspended in 0.1 the original volume. Exonuclease V activity was assayed as described previously by Eichler and Lehman (12). One unit was defined as the amount of enzyme which releases 1 nmol of acid-soluble radioactivity in 20 min at 37°C. ^{3}H-labeled T7 double-stranded DNA was used as the substrate.

DEAE-cellulose chromatography was performed essentially by the method of Eichler and Lehman (12) except that NH_4Cl instead of KCl was used for the salt gradient.

Assay of protease III activity. Periplasmic extracts were prepared as described previously by Nossal and Heppel (34). Assay conditions were those previously outlined by Swamy and Goldberg (39) except that degradation of insulin chain B was determined on 12.5% polyacrylamide gels as described before by Cheng and Zipser (6).

RESULTS

Cloning the *recB-recC* region. Since it has been suggested that amplification of exonuclease V might be lethal to the cell (31), the *recB* and *recC* genes were cloned in a two-stage procedure, using a single-copy plasmid vehicle (pDP3) and λ1059 (21). In the first step, a 3-kb *Sal*I fragment of AB1157 chromosomal DNA carrying the flanking *argA* gene (Fig. 1) was isolated in the low-copy vehicle pDP3. This fragment was subsequently subcloned into pBR322, and the resulting plasmid (pCDK2) was used to probe AB1157 chromosomal DNA (38), which had been digested with restriction endonucleases that cut the 3-kb *Sal*I fragment one or more times. Of most interest was the fact that two *Bam*HI fragments (19.0 and 8.0 kb) hybridized to pCDK2 plasmid DNA (data not shown).

To obtain these *Bam*HI fragments, an *E. coli* genomic library of *Bam*HI inserts in λ1059 was probed (28) with the 3.0-kb *Sal*I fragment as described above. Recombinant phage containing either the 19.0- or 8.0-kb *Bam*HI fragments were obtained. Both fragments were independently transferred into the *Bam*HI site of pBR325. Recombinant plasmids were transformed into JC5743 (*recB21*), with selection for Cm^r Tc^s colonies. Transformants carrying pCDK3 (contains the 19-kb *Bam*HI fragment; Fig. 1) became resistant to UV light. In addition, pCDK3 complemented strains carrying the *recC22*, *recC53*, *recC82*, *recB21*, *recB58*, *recB60*, and *recB95* alleles, as well as various *thyA* and *argA* mutations (data not shown). The restriction map of pCDK3 is shown in Fig. 1. Plasmids containing the 8.0-kb *Bam*HI fragment did not complement either *recB* or *argA* mutations.

Quantitative complementation analysis. Derivatives of AB1157 (*recB*$^+$ *recC*$^+$), JC5743 (*recB21 recC*$^+$), JC5489

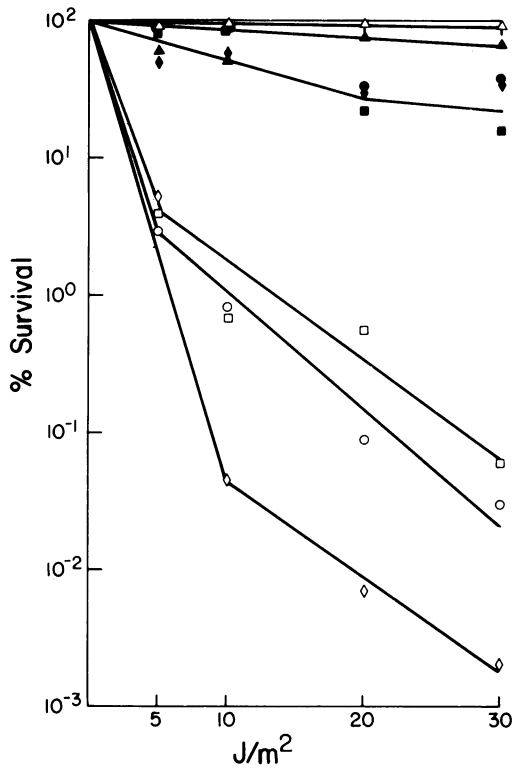


FIG. 2. Analysis of UV resistance of strains with and without pCDK3. UV survival analysis was carried out as described in the text. Symbols: △, SK4544 (*recB*$^+$ *recC*$^+$ [pBR325]); ▲, SK4545 (*recB*$^+$ *recC*$^+$ [pCDK3]); ○, SK4550 (*recB*$^+$ *recC22* [pBR325]); ●, SK4551 (*recB*$^+$ *recC22* [pCDK3]); □, SK4548 (*recB21 recC*$^+$ [pBR325]); ■, SK4549 (*recB21 recC*$^+$ [pCDK3]); ◇, SK4552 (*recB21 recC22* [pBR325]); ◆, SK4553 (*recB21 recC22* [pCDK3]).

(*recB*$^+$ *recC22*), and JC5519 (*recB21 recC22*) containing either pCDK3 or pBR325 were constructed and tested for UV survival as described above. All strains containing pCDK3 exhibited levels of UV sensitivity comparable to those in the wild-type control strain (SK4544) (Fig. 2).

Genomic Southern hybridizations. To determine whether the 19-kb fragment in pCDK3 had been altered during the cloning process, ^{32}P-labeled pCDK3 plasmid DNA was hybridized to chromosomal DNA from *E. coli* K-12 strains of different genetic derivations (Fig. 3). Chromosomal DNA from strains AB1157, BW1001, and C600 after digestion with *Bam*HI, *Sal*I plus *Bam*HI, *Hin*dIII, or *Eco*RI gave identical patterns of hybridization. Chromosomal DNA isolated from strain KL399, however, gave a radically different pattern. In addition to the equally sized bands seen with C600, BW1001, and AB1157 chromsomal DNA, over 17 kb of additional DNA fragments hybridized to the pCDK3 probe (Fig. 3, lanes A5, B5, C5, and D5). The *Eco*RI digest (Fig. 3, lane D5) showed that the secondary region of homology in KL399 was not contiguous, since the primary band (>35 kb) was seen in all four strains (Fig. 3, lanes D2 through D4).

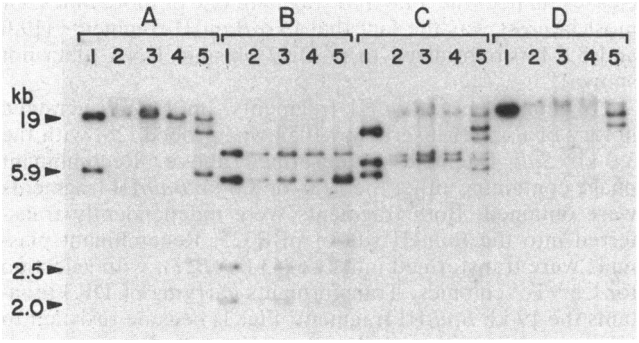


FIG. 3. Southern hybridization of pCDK3 chromosomal DNA. Chromosomal DNAs from a variety of *E. coli* K-12 strains were digested with the restriction enzymes indicated and then run on an 0.8% agarose gel. (A) *Bam*HI; (B) *Bam*HI plus *Sal*I; (C) *Hin*dIII; (D) *Eco*RI. After Southern transfer, the nitrocellulose paper was hybridized with radioactively labeled pCDK3 plasmid DNA. Lanes: 1, plasmid pCDK3; 2, strain AB1157; 3, strain BW1001; 4, strain C600; 5, strain KL399.

Polypeptides encoded by pCDK3. In addition to the plasmid-encoded ampicillin and chloramphenicol resistance determinants, pCDK3 encoded seven distinct polypeptides (Fig. 4, lane 2). Since the 19-kb fragment was cloned into the *Bam*HI site of pBR325, the *tet* gene was inactivated.

The *recB* and *recC* genes have previously been correlated with subunit sizes of ca. 128,000 and 140,000 M_r (12, 14, 17). Marvil and Leisinger (30) purified the *argA* gene product, *N*-acetyl glutamate synthase, and found an M_r of ca. 52,000. Previous work by Hickson et al. (17) and Belfort et al. (2) has established the molecular weight of the *thyA* gene product, thymidylate synthetase, to be ca. 33,000. The structural gene for protease III (*ptr*) has tentatively been mapped between *thyA* and *argA* (1). Cheng and Zipser (6) have determined an M_r of 110,000 for this protein. There were no previously described genetic loci to account for the polypeptides of 22,000 or 50,000 M_r.

Gene localization and direction of transcription. To verify the genetically derived *thyA-recC-recB-argA* gene order (1) and to determine the direction of transcription for each gene,

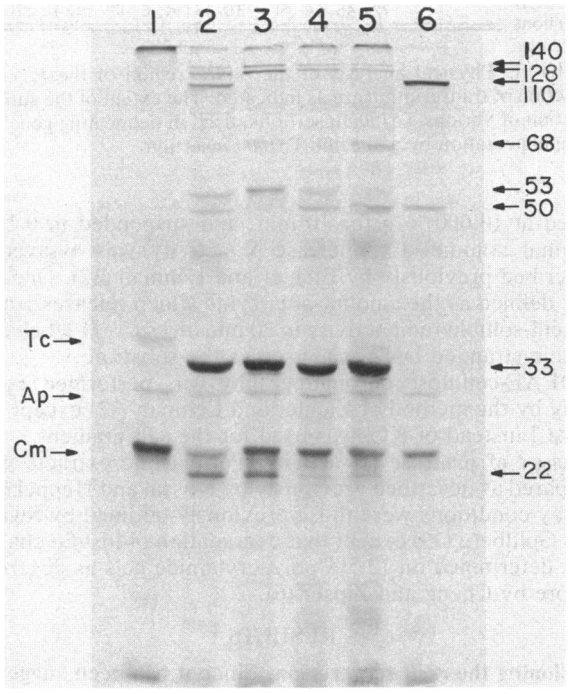


FIG. 4. Maxicell analysis of pCDK3 and its derivatives. Maxicells were prepared as described previously by Sancar et al. (36) and analyzed by sodium dodecyl sulfate gel electrophoresis and autoradiography. Lanes: 1, pBR325; 2, pCDK3; 3, pCDK3::Tn*1000*-24; 4, pCDK3::Tn*1000*-71; 5, pCDK3::Tn*1000*-73; 6, pCDK35. The arrows on the right indicate the seven pCDK3 polypeptides shown in lane 2.

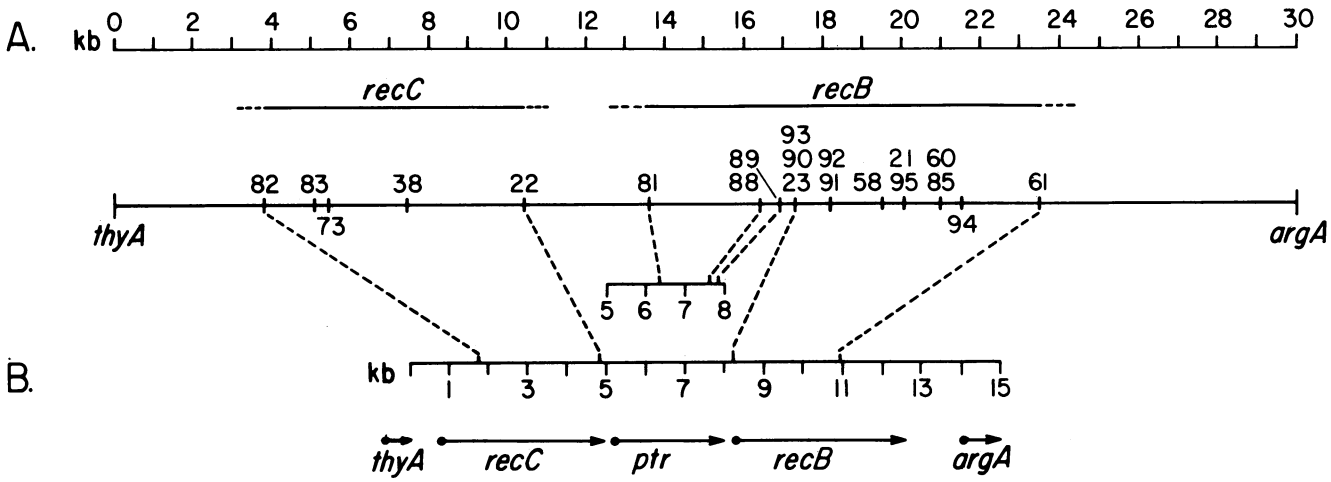


FIG. 5. Correlation of genetic and physical maps. (A) Genetic location of *recB* and *recC* genes in relation to *thyA* and *argA* as determined by the formula of Wu (45); (B) actual physical location of *thyA*, *recC*, *ptr*, *recB*, and *argA*.

a series of transposon Tn*1000* insertions (15) in pCDK3 were generated. In addition, subclones of pCDK3 were constructed which separated *thyA* (pCDK27), *recC* (pCDK4), *recB* (pCDK30), *ptr* (pCDK35), and *argA* (pCDK6) on individual DNA fragments (Fig. 1). These plasmids were shown to complement their respective chromsomal loci either genetically or enzymatically, indicating that each gene is independently transcribed.

The Tn*1000* insertions were mapped to the locations shown in Fig. 1 by restriction enzyme analysis. Insertions 72 and 45 inactivated the ability of pCDK3 to complement *thyA* mutations. Maxicell analysis showed that the 33,000-M_r polypeptide disappeared and that a cryptic peptide of ca. 31,000 M_r was seen with Tn*1000*-45 (data not shown), indicating transcription rightward towards the *recC* gene.

Insertion 57 genetically inactivated *argA* and, when analyzed in maxicells, a cryptic peptide of ca. 51,000 M_r (data not shown) replaced the 53,000-M_r polypeptide that corresponds to the *argA* gene product.

Genetic complementation of the *recC* gene was prevented by insertions 36, 38, 14, 10A, 16, 21, 13, 52, and 64. Cryptic polypeptides were produced in maxicells by insertions 14, 10A, 21, 16, 52, and 13 (data not shown), permitting the localization of the *recC* gene and the determination of its direction of transcription as rightward (Fig. 1). Insertions 1, 10B, 62, 2, 5, 4, 67, 8, 9, 17, 74, 3, and 7 inactivated *recB*, as determined by genetic complementation analysis. Maxicell analysis of these insertions, as well as various subclones, indicated that *recB* was also transcribed rightward (data not shown) (Fig. 1).

Protease III (*ptr*) and p50 (110,000 and 50,000 M_r, respectively) appeared to be related. Tn*1000* insertions which altered *ptr* production in maxicells also changed p50 if they were located in the proximal half of *ptr* (Fig. 4, lane 3). Insertion 71 produced only the 50,000-M_r polypeptide in maxicells (Fig. 4, lane 4), as expected from its location on the restriction map of pCDK3 (Fig. 1). Insertion 73, which gave a cryptic protein of ca. 68,000 M_r, did not affect p50 production (Fig. 4, lane 5). In addition, a subclone (pCDK35) that contains the central 6.8-kb *Hin*dIII fragment from the 19-kb *Bam*HI insert (pCDK3) encoded both *ptr* and p50 but neither *recB* nor *recC* (Fig. 4, lane 6).

The remaining protein encoded by pCDK3, p22 (22,000 M_r), was localized to the extreme left end of pCDK3 by maxicell analysis of various subclones (data not shown) and was not inactivated by any Tn*1000* insertions analyzed to date. Its approximate location is shown in Fig. 1.

DNA sequence homology between *recB* and *recC*. Restriction fragments containing portions of either *recB* or *recC* were eluted from agarose or acrylamide gels and used as hybridization probes against pCDK3 and its subclones digested with a variety of enzymes. No cross-hybridization between the *recB* and *recC* genes was observed (data not shown).

Correlation of genetic and physical maps. Willetts and Mount (44) have published extensive P1 cotransduction data for the *recB*, *recC*, *argA*, and *thyA* loci. When these cotransduction frequencies were converted into map units (45) and then subsequently into kb (1), the relative map positions of a number of *recB* and *recC* alleles were determined (Fig. 5A). If the distances measured by P1 cotransduction were reduced by 53% to match the actual physical distance between *thyA* and *argA*, the majority of the *recB* and *recC* alleles correlated well with the physical map, excepting *recB81*, *recB88*, and *recB89* (Fig. 5B).

Amplification of exonuclease V activity. Assays of ATP-dependent exonuclease activity in Rec^+ and Rec^- backgrounds with and without pCDK3 showed a 30- to 40-fold increase in enzyme activity over wild-type levels (Table 2). No apparent effect on the amount of ATP-dependent exonuclease activity was seen when a strain containing a Tn*1000* insertion in the *ptr* (p50) gene was used (Table 2).

When cell extracts containing pCDK3 were fractionated on DEAE-cellulose, an even larger increase in ATP-dependent exonuclease activity (Fig. 6) was seen, with a specific activity 50-fold higher at this stage of purification than that reported by Eichler and Lehman (12).

DISCUSSION

We cloned the *recB* and *recC* genes of *E. coli* on a 19-kb *Bam*HI fragment by using DNA containing the flanking *argA* locus as a hybridization probe. Although it has been hypothesized that amplification of exonuclease V activity might be lethal to the cell (31), the fragment was readily transferred into the multicopy plasmid pBR325. The fact that Hickson and Emmerson (17) isolated *recB* and *recC* on separate *Bam*HI fragments most likely was due to the cloning procedure they employed which involved initially generating a specialized λ transducing phage. In addition, the results presented in Fig. 3 show that the strain they started with

TABLE 2. Exonuclease V activity in strains carrying pCDK3 and its derivatives[a]

Strain	Genotype	Plasmid	Sp act (U/mg)
SK4552	*recB21 recC22*	pBR322	0
SK4544	*recB*$^+$ *recC*$^+$	pBR322	40
SK4553	*recB21 recC22*	pCDK3	1,260
SK4545	*recB*$^+$ *recC*$^+$	pCDK3	1,610
SK5107	Δ*recB* Δ*recC*	pCDK3::Tn*1000*-24	2,120
SK5108	Δ*recB* Δ*recC*	pCDK3	1,890

[a] Exonuclease V activity was assayed as described in the text.

(KL399; lanes A5, B5, C5, and D5) contains a partial duplication of the *thyA-argA* region. Since the duplicated region has a *Bam*HI site, this could also account for the cloning of *recB* and *recC* on separate *Bam*HI fragments by Hickson and Emmerson (17). Our results show that the duplicated DNA is most likely located outside the normal *thyA-argA* region (Fig. 3).

When the recombinant plasmid (pCDK3) was analyzed in *E. coli* maxicells, seven polypeptides were detected. Five of the proteins of M_r 140,000, 128,000, 110,000, 53,000, and 33,000 were identified as the products of the *recB*, *recC*, *ptr*, *argA*, and *thyA* genes, respectively, on the basis of genetic complementation and enzyme assays (Fig. 1). The observed molecular weights are in good agreement with those reported previously for these proteins (2, 7, 12, 14, 16, 17, 30).

Of particular interest was the finding that both protease III (*ptr*) and p50 were encoded in the region located between *recC* and *recB* (Fig. 3). Whether p50 represents the putative 60,000-M_r α subunit of exonuclease V reported by Lieberman and Oishi (26) will require further study. In addition, A. Karu (unpublished data) has observed a 100,000-M_r protein in preparations of exonuclease V. If either additional polypeptide is required for normal levels of the ATP-dependent exonuclease activity, the results shown in Table 2 would tend to argue against *ptr* (p50) being the structural gene for either protein. Plasmid pCDK3::Tn*1000-24* does not produce either *ptr* or p50 (Fig. 3), yet it yielded more exonuclease V activity than its wild-type parent (Table 2).

Maxicell analysis of a large number of Tn*1000* insertions in pCDK3 has permitted the determination of the location and direction of transcription of *thyA*, *recC*, *ptr* (p50), *recB*, and *argA* (Fig. 1). The preliminary results obtained by S1 nuclease mapping for *recC* and *recB* by Sasaki et al. (37) agree with those described here. Sasaki et al. (37) did not report any data for *thyA* and *argA*, nor did they mention the existence of either p22 or *ptr* (p50). It should also be noted that Eckhardt (11) examined some *argA* transducing phages and determined a direction of transcription which is opposite to that described here. The reason for this discrepancy is not known.

Another very interesting aspect of the physical mapping of the *recB* and *recC* region is that *recC*, *ptr* (p50), and *recB* are contiguous and transcribed in the same direction but appear to be transcribed independently. It is worth noting that the flanking *thyA* and *argA* loci are both separated by at least 1 kb from the start of *recC* and the end of *recB*. Essentially all of the 19 kb of DNA in pCDK3 is transcribed and translated.

Relative distances on the *E. coli* genome (1) have been derived by converting P1 cotransduction frequencies into map units by the formula derived by Wu (45). When the cotransduction data of Willetts and Mount (44) were converted to map units and subsequently to kb, a 53% reduction generated a genetic map which correlated well with the actual physical distances (Fig. 5). Of note, however, is the fact that *recB81*, *recB88*, and *recB89* appeared to map in *ptr* (p50). In addition, there seemed to be no mtuations in the distal portion of the *recB* gene. This analysis again confirms that whereas P1 cotransduction is good for ordering genes, it is not accurate for determining distances between genes.

Not only was it straightforward to transfer the 19-kb *Bam*HI fragment carrying *recB* and *recC* into a multicopy plasmid, but the data described in Table 2 and Fig. 6 demonstrate that cells carrying such a plasmid (pCDK3) produce anywhere between 30- and 50-fold-more exonuclease V. Furthermore, such strains of *E. coli* did not exhibit any change in growth rate relative to that of wild-type controls (data not shown) and appeared nearly as resistant to UV light as did wild-type controls (Fig. 2). Thus, overproduction of exonuclease V does not appear to alter the ability of the cell to respond to DNA damage, as has been observed with the overproduction of either the *uvrD* (29) or the *ssb* (5) gene product. Furthermore, the amplification of the enzyme should be very helpful in developing a simpler purification procedure for exonuclease V. In addition, it will also be possible to generate new temperature-sensitive *recB* and *recC* mutations by in vitro mutagenesis of the cloned gene.

Finally, it is interesting to speculate why the structural gene for protease III has been sandwiched between *recB* and *recC*. Our data suggest that both *ptr* and p50 originate from the same region of DNA but do not prove that they are translated from the same reading frame. Furthermore, preliminary results (data not shown) indicate that both proteins are located in the periplasm. If one takes into account that protease III is known only to have Mg^{2+}-dependent endopeptidase activity on small (<6,000 M_r) polypeptides (39), it is not apparent why the three genes exist as a cluster. It is possible that protease III may be involved in the regulation of exonuclease V activity by means of some specific proteolytic event. Alternatively, the enzyme may also have some nucleolytic activity. At this point, no catalytic activity has been associated with p50. Clearly, this question deserves further study.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grants GM27997 and GM28760 from the National Institutes of Health.

C.C.D. was a recipient of a National Institutes of Health predoctoral fellowship.

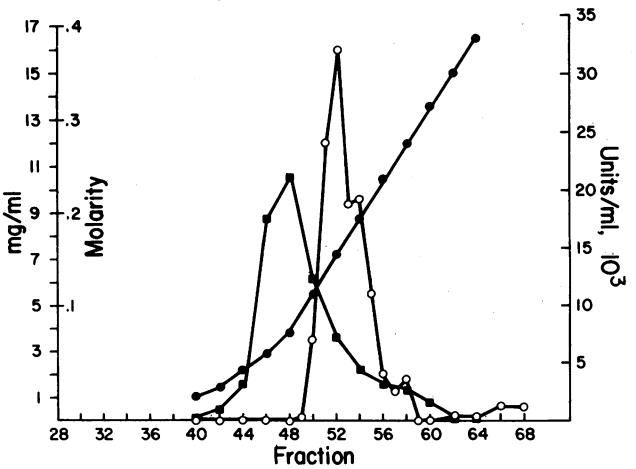


FIG. 6. DEAE-cellulose elution profile of exonuclease V activity from SK4545. Symbols ■, Protein concentration (milligrams per milliliter); ●, NH_4Cl, molarity; and ○, exonuclease V activity (10^3 units per milliliter).

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