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Genetics

recD: The gene for an essential third subunit of exonuclease V

[genetic recombination/cell viability/nuclease-negative, recombination-positive (*recB*‡) phenotype/RecBCD enzyme]

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ABSTRACT **Exonuclease V (EC 3.1.11.5) of *Escherichia coli*, an enzyme with multiple activities promoting genetic recombination, has previously been shown to contain two polypeptides, the products of the *recB* and *recC* genes. We report here that the enzyme contains in addition a third polypeptide (α) with a molecular mass of about 58 kDa. The α polypeptide is not synthesized by a class of mutants (previously designated *recB*‡) lacking the nuclease activity of exonuclease V but retaining recombination proficiency. The gene, *recD*, coding for the α polypeptide is located near *recB* in the order *thyA–recC–ptr–recB–recD–argA* on the *E. coli* chromosome. The *recB* and *recD* genes appear to be governed by a common promoter to the left of *recB*; a weaker promoter appears to govern *recD* alone. In the light of these results we discuss the relation between the structure and function of the three polypeptides of exonuclease V, hereby alternatively designated RecBCD enzyme.**

Exonuclease V (EC 3.1.11.5) of *Escherichia coli*, often designated ExoV or RecBC enzyme, is a complex enzyme possessing multiple enzymatic activities and participating in numerous cellular functions (for review, see ref. 1). ExoV binds to double-stranded DNA (ds DNA) ends and then rapidly moves along the DNA, unwinding it and producing single-stranded loops (2, 3). During this unwinding, which requires ATP hydrolysis, the enzyme cuts the DNA and releases single-stranded DNA (ss DNA) fragments (4, 5). Cutting occurs with high frequency at Chi sites, 5′-G-C-T-G-G-T-G-G-3′, which stimulate genetic recombination in their vicinity (6, 7). ExoV can also hydrolyze ss DNA; linear ss DNA is hydrolyzed about 500 times more rapidly than circular ss DNA (8). These nuclease activities, like unwinding, are coupled to ATP hydrolysis.

ExoV plays a central role in homologous genetic recombination, recovery from DNA damage, maintenance of cell viability, and the destruction of damaged and foreign DNA (1). The role that each of the enzymatic activities plays in these cellular functions is understood in some cases but not in others. The results of *in vitro* and *in vivo* studies have indicated that the DNA unwinding and the Chi-dependent DNA cutting activities are important for homologous recombination (6, 7, 9); it has been proposed that the combined unwinding and Chi cutting activities generate a ss DNA tail that is efficiently synapsed with homologous ds DNA by RecA and ss DNA-binding (SSB) proteins (10). These activities of ExoV may play a similar role in the recovery from DNA damage. The destruction of damaged and foreign DNA requires one or more nuclease activities of ExoV (9). The basis for the requirement for ExoV in the maintenance of cell viability is not clear, but the nuclease activity does not seem to be required (9, 11); the unwinding activity of ExoV may aid chromosome segregation.

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The subunit composition of ExoV has been uncertain. Genetic complementation analysis of ExoV null mutants (those lacking any detectable activity) revealed two genes, *recB* and *recC* (12). These genes code for two polypeptides with approximate molecular masses of 120 and 110 kDa, respectively, seen in purified preparations of ExoV (8, 13, 14). ExoV can be dissociated by high salt and separated by column chromatography into two inactive fractions, designated α and β; mixing α and β restores nuclease and ATPase activity (15, 16). Unexpectedly, α activity is present in extracts of both *recB* and *recC* null mutants, while β is present in neither. Apparently β contains the *recB*- and *recC*-encoded polypeptides, and α contains the product of an unidentified gene.

A special class of ExoV mutants, designated *recBC*‡, lacks detectable ExoV nuclease activity (9). Unlike *recB* and *recC* null mutants, the *recBC*‡ mutants are recombination proficient (but Chi nonactivating), resistant to DNA-damaging agents, and fully viable; we have speculated that these mutants retain the DNA-unwinding activity of ExoV (9). We report here that the *recB*‡ mutants lack the α subunit of ExoV, a polypeptide of about 58 kDa encoded by the gene *recD*, which is located near *recB* and defined in this paper.

MATERIALS AND METHODS

Bacteria and plasmids are described in Tables 1 and 2, Fig. 1, or ref. 18. Culture media, phage growth tests, phage P1 transductions, conjugations, and RecBCD nuclease assay were as described (18). Tn*1000* insertions into plasmids were isolated as described (27) by mating strain V767 with strain V186. Labeling and analysis of plasmid-encoded polypeptides in "maxicells" were essentially as described (24).

Purification of ExoV. ExoV was prepared from cells (200 g wet weight) of strain V319 obtained from 15 liters of culture grown at 41°C to late logarithmic phase in supplemented L broth (6). Cells were lysed and the enzyme was purified as described (6, 13) through the agarose column chromatography step, yielding as fraction IV about 66 mg of protein with a specific activity of 3.6×10^4 units/mg. Three-eighths of fraction IV was chromatographed on heparin-agarose (3), yielding as fraction V about 2 mg of nearly homogeneous enzyme (Fig. 2) with a specific activity of 3×10^5 units/mg. ExoV was similarly prepared from strain AFT380 carrying the *thyA–argA* region on the F′15 factor (6).

Preparation of α and β Fractions of ExoV. ExoV (30,000 units of fraction V) from strain V319 was diluted into buffer Q (20 mM K-PO_4, pH 7.6/0.1 mM EDTA/50 mM dithiothreitol) and concentrated with an Amicon Centricon-30 microconcentrator. Solid NaCl was added to 4 M. After incubation

Abbreviations: ExoV, exonuclease V; ss DNA, single-stranded DNA; ds DNA, double-stranded DNA; kb, kilobase pair(s).

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Table 1. Bacterial strains

Strain	Genotype	Ref. or source
V186	*Δ(argA–thyA)232* IN(*rrnD–rrnE*)*1*	Strain AC113 (ref. 17)
V197	*thr-1 leuB6 thi-1 lacY1 tonA21 supE44 recA56 srl-300*::Tn*10*	AFT140 × C600 (ref. 18)
V220	*recD1011 recF143 hisG4 met rpsL31 argA21*	Ref. 9
V319	*thyA tonA his gal rpsL endA supE* (pDWS103)	Transf. of AFT379 (ref. 6)
V408–V412	As in V186 plus (pAC1)–(pAC5)	Transf. of V186
V767	*leuB6 hisG1 recA1 argG6 metB1 lacY1 gal-6 malA1 xyl-7 mtl-2 rpsL104 tonA2 supE44* (F'131 *his*$^+$) (pDWS2)	Transf. of V156 (ref. 18)
AFT380	*thyA tonA his gal rpsL endA supE* (F'15 *thyA*$^+$–*argA*$^+$)	Ref. 6

Additional *E. coli* and phage strains used are described in ref. 18. A × B, P1-mediated transduction in which A is the donor and B is the recipient; Transf., transformation.

at 0°C for 2.5 hr, the solution was diluted with an equal volume (100 μl) of buffer Q containing 2 M NaCl and loaded onto a 13-ml 10–30% glycerol gradient in buffer Q containing 3 M NaCl. Centrifugation was at 70,000 rpm for 19 hr at 5°C in a Beckman type 70.1 Ti rotor. Fractions containing β activity were initially identified by polyacrylamide gel electrophoresis; these pooled fractions were used to identify fractions containing α activity by the complementation assay described below. Subsequent assays, such as that shown in Fig. 2 *Left*, used these pooled fractions to identify α and β by the complementation assay. The yields of α and β activity were severalfold higher when bovine serum albumin (Metrix, Armour Pharmaceuticals, Chicago) was present at 1 mg/ml in the high-salt buffer and in the glycerol gradient.

***In Vitro* Complementation.** Reactions were in buffer R [buffer Q containing 100 mM NaCl, 10% (vol/vol) glycerol, and bovine serum albumin at 1 mg/ml]. In reactions with purified α or β fractions 1 or 2 μl of each fraction was mixed in a final volume of 10 μl, incubated for several hours (or overnight) at room temperature, and assayed for ATP-de-

Table 2. Plasmids

Plasmid	Genotype of insert	Vector	Ref. or source
pAC1	*recD1009*	pBR322	V324
pAC2	*recC1010*	pBR322	V375
pAC3	*recD1011*	pBR322	V768
pAC4	*recD1013*	pBR322	V323
pAC5	*recD1014*	pBR322	V355
pAFT12	Wild-type	pUC18	pDWS2
pAFT13	Wild-type	pUC18	pDWS2
pDWS2	Wild-type	pBR322	Ref. 6
pDWS103	Wild-type	pMOB45	pDWS2

Additional plasmids are described in Fig. 1. Except for pDWS103, pAFT12, and pAFT13, all plasmids in this table contain the 18.5-kilobase-pair (kb) *Bam*HI *thyA–argA* fragment of *E. coli* DNA (19) inserted into the *Bam*HI site of pBR322. Plasmids pAC1–pAC5 were constructed from pBR322 as described (6) by inserting *Bam*HI fragments from the indicated strains [thymine-independent transductants of strain 594 *thyA* using P1 phage grown on strains V218–V223 (ref. 9)]. Plasmid pDWS103 contains the large *Bam*HI fragment from pDWS2 inserted into the *Bam*HI site of pMOB45 (ref. 20). Plasmid pAFT12 contains the 3.6-kb *Pst* I–*Pst* I fragment from 14.3 to 17.9 kb (see Fig. 1) inserted into the *Pst* I site of pUC18 (ref. 21) with the *argA* end nearer the *Hind*III site of pUC18. Plasmid pAFT13 contains the same fragment in the opposite orientation. These orientations were determined by comparisons of *Sal* I digests of pAFT12 and pAFT13 with *Pst* I digests of the *Sal* I–*Sal* I (13.9–16.5 kb in Fig. 1) and *Sal* I–*Bam*HI (16.5–18.5 kb) fragments purified from pDWS2. Dykstra *et al.* (22) reported a *Pvu* II and an *Ava* I site in the *Pst* I–*Sal* I segment (14.3–16.5 kb) and neither site in the *Sal* I–*Pst* I segment (16.5–17.9 kb). *Sal* I–*Pst* I subclones of pAFT12 and pAFT13 containing these segments confirmed that the *Pvu* II and *Ava* I sites are in the 16.5- to 17.9-kb segment.

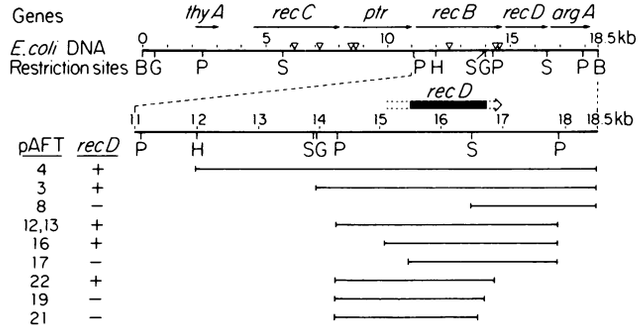

FIG. 1. Genetic and physical maps of the *thyA–argA* region of the *E. coli* chromosome and of plasmids containing portions of this region. The locations of genes, transcripts, and restriction enzyme cleavage sites are from refs. 19, 22, and 23 and our unpublished data. The approximate locations of the Tn*1000* insertions (▽; left to right: *C1029, C1032, ptr-202, ptr-201, B1019, B1016*, and *B1023*) were determined by restriction enzyme cleavage of plasmid DNA with *Pst* I (P), *Sal* I (S), and *Eco*RI. Other cleavage sites are *Sph* I (H), *Bgl* II (G), and *Bam*HI (B). The gene functions encoded by plasmids pAFT3–pAFT22 were determined by genetic complementation of chromosomal mutations *thyA, recC73, recB21, recD1011*, and *argA81*::Tn*10* in derivatives of strain 594 or V66 and by "maxicell" analysis of labeled polypeptides (ref. 24; Figs. 3 and 4). *thyA* gene function was determined only by complementation and *ptr* only by maxicell analysis (see Fig. 4). All were Thy$^-$, RecC$^-$, Ptr$^-$, and RecB$^-$. pAFT3–pAFT8 were ArgA$^+$; the others were ArgA$^-$ (data not shown). pAFT3–pAFT8 were derived from pDWS2 by cleavage with restriction enzymes at the sites indicated and ligation (25); a bar indicates the DNA remaining. pAFT3 contains ≈0.5 kb from the left end of the map in addition to that shown. pAFT16 and pAFT17 were derived from pAFT12 (and pAFT19, pAFT21, and pAFT22 from pAFT13) by partial exonuclease III digestion (ref. 26) after cleavage with *Bam*HI and *Sst* I. After transfer to strain V220, *recD* mutants were identified by their sensitivity to phage T4 *2*$^-$ (ref. 9).

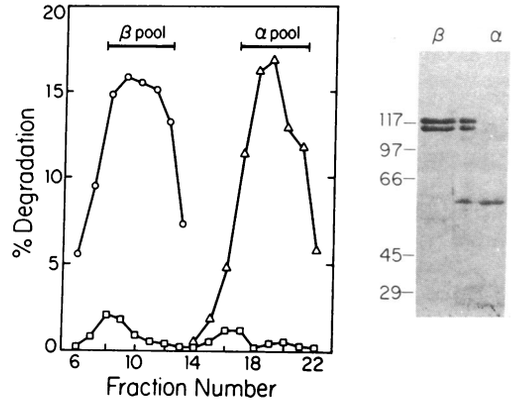

FIG. 2. Polypeptides in purified ExoV preparation and in the separated α and β fractions. (*Left*) Fraction V enzyme from strain V319 was dissociated with 4 M NaCl, sedimented (from right to left) through a glycerol gradient, and assayed for α and β activity. ○, β activity; △, α activity; □, ExoV activity (i.e., without additions). (*Right*) Aliquots (150 μl) of each pool on the left, with 0.25% sodium deoxycholate as carrier, were concentrated by precipitation with trichloroacetic acid. The precipitates were rinsed with cold acetone, dissolved in gel loading buffer, and electrophoresed on a 10% polyacrylamide/NaDodSO$_4$ gel together with 250 units of fraction V (center lane) and markers (SDS-6H mix from Sigma) whose molecular masses in kDa are indicated. The gel was strained with Coomassie blue and photographed. β, β fraction; α, α fraction.

pendent ds DNA exonuclease activity (13). Extracts (≈10–20 mg/ml) of plasmid-bearing cells were prepared (6) and mixed with purified fractions, both diluted in buffer R. Renaturation mixtures typically contained, in 10 μl, 0.8 μg of extract protein and excess purified α or β fraction and were incubated overnight at room temperature before assay.

Note on *rec* Genetic Nomenclature. The designation *recD* was initially reserved (28) for a recombination-deficient mutation, *rec-34* (29), that was later shown to be a *recA* allele (30). Thus, there are no previously described mutations in the *recD* gene (B. J. Bachmann and A. J. Clark, personal communications), which has been assigned for the mutations *rec-1009*, *rec-1011*, *rec-1013*, and *rec-1014* discussed here. We have not further investigated the *rec-1012* (*B*‡) mutation and hence do not know whether it is in *recB* or *recD*.

RESULTS

Three Major Polypeptides in ExoV Preparations. During purification of ExoV from an overproducing *E. coli* strain (V319) we observed three major polypeptides in those fractions from the heparin-agarose affinity column (fraction V) containing ExoV activity: the two large polypeptides observed previously (8, 13, 14) and a polypeptide of about 58 kDa (Fig. 2 *Right*). The novel polypeptide was also observed at the same stage of purification of the enzyme from a nonoverproducing strain (AFT380); although there were several polypeptides present in this fraction, a polypeptide of 58 kDa clearly eluted coincident with ExoV activity from the column (data not shown). The 58-kDa polypeptide also remained associated with the larger polypeptides during sedimentation of fraction V through a glycerol gradient (data not shown). The results below demonstrate that the 58-kDa polypeptide is essential for the nuclease activity of ExoV.

Separation of ExoV into Two Fractions and Its Reconstitution. Following the rationale of Lieberman and Oishi (15, 16), we inactivated ExoV (fraction V) by incubation in a solution with high salt concentration and separated the inactivated enzyme into two fractions by sedimentation on a glycerol gradient (Fig. 2 *Left*). The fractions yielded nuclease activity upon mixing and appeared to behave similarly to the two fractions separated by DEAE-Sephadex chromatography by Lieberman and Oishi (15). We therefore equate our faster-sedimenting fraction with fraction β and the slower one with fraction α of Lieberman and Oishi (15). The β fraction contained the 120- and 110-kDa polypeptides as the sole visible constituents, while the α fraction contained the 58-kDa polypeptide as the sole visible constituent (Fig. 2 *Right*). We infer that the 58-kDa polypeptide is the active species in the α fraction and that it is essential for the reconstitution of salt-inactivated nuclease activity.

***recB*‡ Mutants Lack α Activity; a *recC*‡ Mutant Lacks β Activity.** Since *recBC*‡ mutants lack ExoV nuclease activity (9), we tested whether they lack the activity of one or the other fraction of ExoV. Incubation of extracts of *recB*‡ mutants (Table 3) with purified α fraction resulted in the appearance of ATP-dependent ds DNA exonuclease activity, while incubation with β fraction produced no significant increase in this activity. Conversely, extracts of the *recC*‡ mutant (*recC1010*) produced ATP-dependent ds DNA exonuclease activity with the β fraction but not with the α fraction.

***recB*‡ Mutants Lack the 58-kDa Polypeptide.** To search for a structural defect accounting for the lack of activity in the *recB*‡ mutants, we examined synthesis of polypeptides encoded by plasmid-borne genes by the "maxicell" technique (24). Plasmid pDWS2, with wild-type *E. coli* genes from the *thyA–argA* region (6, 19), directed the synthesis of five major large polypeptides not observed with the parent plasmid pBR322 (Fig. 3). Four of these are the products of the *recB*, *recC*, *ptr*, and *argA* genes (14, 19, 22). The fifth polypeptide was observed with *rec*$^+$ and *recC*‡ plasmids but not with *recB*‡ plasmids. This fifth polypeptide, with molecular mass about 58 kDa, comigrated with α polypeptide of fraction IV enzyme during polyacrylamide/NaDodSO$_4$ gel electrophoresis (Fig. 3). Plasmid pAC1, with the *recD1009* allele, directed the synthesis of a polypeptide with molecular mass of about 41 kDa (Fig. 3). This polypeptide had peptides in common with the 58-kDa polypeptide, as revealed by analysis of partial V8 protease digestion products by polyacrylamide/NaDodSO$_4$ gel electrophoresis (ref. 32; data not shown). Similarly, plasmids with the *recD1013* and *recD1014* mutations directed the synthesis of polypeptides with molecular mass of about 29 kDa (data not shown). We infer from these results that the *recD* mutations *1009*, *1011*, *1013*, and *1014* are nonsense mutations. Full-length α polypeptide was

Table 3. α and β activity in *recBC*‡ mutants

Extract of strain	*rec* allele	ATP-dependent ds DNA exonuclease activity*			Specific activity,† units/mg	
		—	+α	+β	+α	+β
V408	*D1009*	6	26	9	500	48
V410	*D1011*	2	21	2	490	−14
V411	*D1013*	0	15	2	380	28
V412	*D1014*	1	21	2	510	12
V409	*C1010*	0	3	15	34	380

*Extracts were prepared, incubated with α or β fraction (or with neither) as described in *Materials and Methods*, and assayed for ds DNA exonuclease activity with or without 25 μM ATP. Data are the percentages of the labeled DNA (430 pmol of DNA nucleotide in 25 μl) rendered trichloroacetic acid soluble in the assays (20 min at 37°C) with ATP minus the percentages without ATP. The latter values were 2 (or 4 with V410 extract alone). The values for α and β fractions alone were 1 with ATP, 0 without ATP. When mixed, the values for α and β were 24 with ATP, 0 without ATP.

†Units of ATP-dependent ds DNA exonuclease produced per mg of protein in the extract, after subtracting the ATP-dependent activity in the extract alone plus that in the α or β fraction alone. Extracts of strains carrying plasmid pDWS2 typically have specific activities of about 500 units/mg.

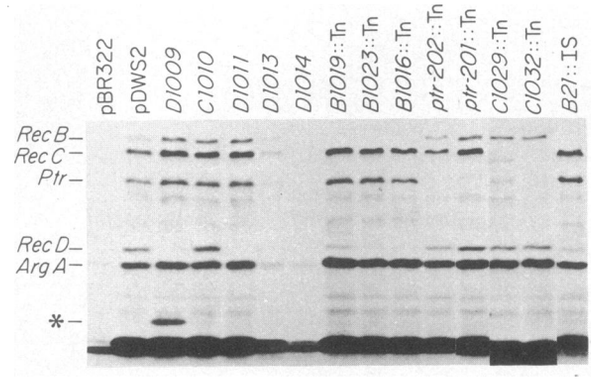

FIG. 3. Polypeptide synthesis in RecBC‡ and Tn*1000* insertion mutants. Proteins from derivatives of strain V197 containing derivatives of plasmid pDWS2 with the indicated *rec* and *ptr* mutations were labeled with [^{35}S]methionine and analyzed by electrophoresis and autoradiography. Lines indicate the positions of the RecB, RecC, and RecD polypeptides of fraction IV enzyme from strain V319 run on the same gel and stained with Coomassie blue (see Fig. 2 *Right* for comparison). Other lines indicate the positions of Ptr and ArgA polypeptides (22, 34). An asterisk indicates a putative truncated polypeptide from the *recD1009* strain. The lanes with the *recC*::Tn*1000* mutations and with the *recB21*::IS mutation were from separate gels; the RecB and RecC polypeptides of the the three gels have been aligned.

synthesized by nonsense-suppressing cells containing these *recD* mutations on plasmids (data not shown).

These results, coupled with the absence of α activity in *recB*‡ mutants, indicate that the 58-kDa polypeptide is α and is the product of the gene in which the *recB*‡ mutations lie. The following results show that this gene is located between *recB* and *argA* but is distinct from them; we designate this gene *recD*.

Location of the *recD* Gene. To test whether the α polypeptide derives from the *recB*, *recC*, or *ptr* genes, we examined polypeptide synthesis directed by plasmids bearing Tn*1000* insertion mutations in these genes (Fig. 3). Insertions in the *recC* and *ptr* genes eliminated synthesis of the 110- and 92-kDa polypeptides, respectively, but did not significantly alter α polypeptide synthesis; insertions in the *recB* gene eliminated synthesis of the 120-kDa polypeptide and reduced α polypeptide synthesis to about 1/5th. The *recB21* mutation is an insertion of about 1.4 kb (data not shown) that is also polar on *recD* (Fig. 3). Partial V8 protease digestion of the reduced level α polypeptide produced peptides comigrating during electrophoresis with those from wild-type level α polypeptide (data not shown). These results indicate that the gene for α polypeptide is separate from *recB*, *recC*, and *ptr* and suggest that *recB*::Tn*1000* insertions are polar on this separate gene (see below).

To locate the *recD* gene, we generated deletion derivatives of the 18.5-kb *Bam*HI fragment in plasmid pDWS2 (Fig. 1). Deletions in plasmids pAFT4 and pAFT3 removing the *thyA*, *recC*, *ptr*, and part of the *recB* genes left *recD* and *argA* functional (Figs. 1 and 4). A more extensive deletion, in pAFT8, abolished *recD* but not *argA* function. These results locate *recD* between 13.95 and 16.5 kb on the *Bam*HI fragment (Fig. 1).

The above results and the apparent polarity of *recB* insertions on the *recD* gene suggest that *recD* is located distal to the *recB* gene, which is transcribed toward *argA* (19, 22). We located *recD* to this region by cloning, in both orientations, a 3.6-kb *Pst* I–*Pst* I DNA fragment from the *recB–argA* region in plasmid pUC18 (ref. 21). Both of these plasmids (pAFT12 and pAFT13) complemented the *recD1011* mutation (Fig. 1) and directed the synthesis of the 58-kDa RecD polypeptide (Fig. 4). These results indicate that the entire *recD* gene, including a promoter for it, is contained within this 3.6-kb segment.

To more precisely locate the *recD* gene, we generated deletions from each end of the *Pst* I–*Pst* I fragment by partial exonuclease III digestion (26). These deletion derivatives were analyzed by genetic complementation (Table 1) and by

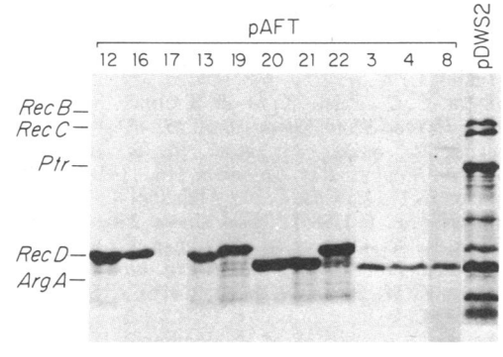

FIG. 4. Effect of deletions on *recD* polypeptide synthesis. Proteins from derivatives of strain V197 containing the indicated plasmids (see Fig. 1) were labeled with [^{35}S]methionine and analyzed as in Fig. 3. Two gels, run for slightly different times, were aligned at the position of the RecD polypeptides; lanes with pAFT3 and those to its right were from one gel, and the lanes to the left of pAFT3 were from the other.

"maxicell" analysis (Fig. 4). Deletions from the *thyA* end of the *recD* gene either left *recD* complementation and α synthesis intact (pAFT16) or abolished both (pAFT17). Deletions from the *argA* end of the *recD* gene either left complementation and α synthesis intact (pAFT22) or abolished complementation and resulted in elongated α polypeptide (pAFT19) or shortened α polypeptide (pAFT21) (Figs. 1 and 4). These results confirm the direction of transcription of *recD* (toward *argA*), demonstrate that *recD* is the structural gene for α polypeptide, and localize *recD* to an ≈1.6-kb segment between *recB* and *argA*.

DISCUSSION

The results reported here identify a gene, *recD*, located between the *recB* and *argA* genes on the *E. coli* chromosome and coding for a polypeptide of about 58 kDa. The RecD polypeptide is an essential part of ExoV: (*i*) separation of the RecD polypeptide from the RecB and RecC polypeptides, two other essential polypeptides of ExoV, results in loss of ExoV nuclease activity, which can be recovered upon mixing the three polypeptides (refs. 15 and 16; Fig. 2; Table 3); and (*ii*) *recD* mutations abolish both the synthesis of the 58-kDa polypeptide and ExoV nuclease activity (ref. 9; Fig. 3; Table 3). On the basis of these observations we propose "RecBCD enzyme," rather than "RecBC enzyme," as an alternative designation for ExoV.

Mutations inactivating the *recD* gene include nonsense mutations and deletions (Figs. 1, 3, and 4). Biek and Cohen (31) have independently isolated *recD* mutations (Tn*10* insertions) based upon a phenotype–plasmid instability—different from those we have used. When compared by phenotypes and genetic location, their mutations and ours are in the same gene. The nonsense mutations *recD1009*, *recD1011*, *recD1013*, and *recD1014* were previously designated as *recB* alleles on the basis of their failure to complement the commonly used *recB21* allele (9). We found, unexpectedly, that *recB21* is an insertion of about 1.4 kb (data not shown) that is polar on *recD* (Fig. 3). This polarity accounts for the lack of complementation. On the basis of the observations reported here, the *recB*‡ alleles listed above are redesignated *recD* alleles.

Our results are in accord with previous reports on the subunit composition and genetics of RecBCD enzyme. Dykstra *et al.* (33) reported that purified preparations of ExoV from an overproducing strain contained three polypeptides with molecular masses similar to those of our preparation (Fig. 2 *Right*). The RecD polypeptide is the α subunit shown by Lieberman and Oishi (15) to be present in *recB* and *recC* mutant extracts. In accord with our finding that *recB21* reduces RecD polypeptide synthesis to about 1/5th (Fig. 3), they reported about 10% as much α activity in *recB21* extracts as in *recC22* extracts (15). As suggested by Lieberman and Oishi (15, 16), the RecD polypeptide (α subunit) is necessary for nuclease activity but not for recombination proficiency or resistance to DNA-damaging agents such as UV light (9). These observations provide an explanation for the failure to find *recD* mutants in previous searches that assayed for recombination deficiency or sensitivity to DNA-damaging agents (12). We believe that the RecD polypeptide may have not been detected previously (8, 13) due to the small amounts of protein available for assay. Two groups have reported reconstitution of ExoV nuclease activity, using cloned *recB* and *recC* genes. Umeno *et al.* (34) reconstituted ExoV from crude extracts of cells containing plasmids with the *recB* and *recC* genes; their *recB* plasmid contained the chromosomal segment in which we have located *recD*. Hickson *et al.* (35) reconstituted ExoV from RecB and RecC polypeptides prepared from cells containing plasmids with the *recB* and *recC* genes; we presume that their *recB* clone contains *recD* and that a small amount of RecD

polypeptide in their RecB preparation accounts for the low specific activity, compared to that of the native enzyme (ref. 13; *Materials and Methods*), of ExoV reconstituted from their RecC and RecB preparations. Finally, the *Haemophilus influenzae* ExoV is also composed of three subunits with sizes similar to those of the *E. coli* enzyme (36), to which it is functionally similar (1, 2, 37).

Our results suggest that the *E. coli recB* and *recD* genes form an operon, with a major external promoter for both genes and a minor internal promoter for *recD*. This conclusion stems from the reduction, but not abolition, of *recD* expression by *recB* insertions and deletions (Figs. 1, 3, and 4). An alternative explanation, that RecB polypeptide is required for high-level expression of the *recD* gene, is argued against by the failure of *recB21* (*recD*$^+$) to be complemented by (*recB*$^+$) *recD* mutations (9). A few other *E. coli* operons, for example *glnALG* (38) and *rpsU–dnaG–rpoD* (39), have a similar arrangement of promoters in which the internal promoter allows in certain physiological conditions discoordinately high levels of the "downstream" gene products; such a condition for *recD* is not evident at present.

What are the functions of the three polypeptides of RecBCD enzyme? Hickson *et al.* (35) have reported that the isolated RecB polypeptide has DNA-dependent ATPase activity, which we calculate to be equivalent to that of holoenzyme. The β fraction, composed of the RecB and RecC polypeptides, has, however, only about 5–10% as much ATPase activity as holoenzyme (15). The ATPase activity may reside in the RecB polypeptide but may be fully functional only when the RecD polypeptide is present. One class of *recC* mutants, designated *recC**, retains all tested activities except Chi activation (6, 18). Recognition of the Chi sequence may reside in the RecC polypeptide. The results reported here indicate that the nuclease activity requires the RecD polypeptide, in which the nuclease activity may reside. Since *recD* mutants retain recombination proficiency, resistance to DNA-damaging agents, and high cell viability (9), the combined RecB and RecC polypeptides must retain some activity. We have suggested that this is the DNA-unwinding activity (9). It must be kept in mind, however, that any one of the activities may require the proper association of two, or all three, polypeptides. This possibility is strongly supported by the lack of nuclease activity in the *recC1010* mutant as well as in the *recD* mutants and by the lack of nuclease activity in isolated α and β fractions. Evidently the nuclease activity requires proper interaction between the RecD and RecC (and possibly RecB) polypeptides. Additional correlations of functions with the separate polypeptides of RecBCD enzyme are likely to provide further insight into the mechanism by which this complex enzyme promotes genetic recombination, recovery from DNA damage, and maintenance of cell viability.

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