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Genetic and Molecular Analyses of the C-Terminal Region of the *recE* Gene from the Rac Prophage of *Escherichia coli* K-12 Reveal the *recT* Gene

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The nucleotide sequence of the C-terminal region of the *recE* gene of the Rac prophage of *Escherichia coli* K-12 reveals the presence of a partially overlapping reading frame we call *recT*. Deletion mutations show that *recT* is required for the RecE pathway of conjugational recombination. By cloning *recT* with a plasmid vector compatible with pBR322, we showed by *cis-trans* tests that the portion of the *recE* gene encoding ExoVIII DNA nuclease activity is also required for RecE pathway conjugational recombination. The *recT* gene can replace the *redB* gene of lambda for *recA*-independent plasmid recombination. A Tn*10* insertion mutation previously thought to be in *recE* is located in *recT* and is renamed *recT101*::Tn*10*. Discrepancies between the molecular mass estimates of wild-type ExoVIII protein determined from mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and calculated from the predicted amino acid sequence are discussed. The hypothesis that wild-type ExoVIII protein results from fusion of RecE and RecT proteins is disproved genetically, thus supporting a previous hypothesis that the discrepancies are due to abnormal protein mobility in SDS-PAGE. A computer-performed scan of the bacteriophage nucleotide sequence data base of GenBank revealed substantial similarity between most of *recE* and a 2.5-kb portion of the b2 region of lambda. This suggests interesting speculations concerning the evolutionary relationship of lambda and Rac prophages.

recE was discovered by Barbour et al. (2) as a gene encoding an ATP-independent DNA exonuclease, later called ExoVIII (27). This RecE nuclease was purified by two groups (19, 23, 26) and found to be a protein with a subunit molecular mass estimated to be 120 or 140 kDa. The *recE* gene was therefore anticipated to contain an open reading frame (*orf*) approximately 4 kb in length (19). Gillen et al. (19) also hypothesized that RecE protein might have multiple functions, namely, the nuclease function encoded by the lambda gene *redX* and the function, unknown at the time, of the beta protein encoded by the lambda gene *redB*. Both *redX* and *redB* functions are required for lambda recombination (33, 37). In 1989 the nucleotide sequence of about 2.1 kb of *recE* was published (10). That work was performed to characterize two types of deletion mutations which had occurred in *recE*: internal deletions and external deletions (40). Internal deletions removed a large portion of the gene fusing the translational start sequence of *recE* to another portion of *recE* downstream. External deletions fused the translational start sequence of upstream gene *racC* to the remainder of *recE*. Each of these deletion mutations produced a nuclease activity corresponding to that of wild-type RecE protein, but the molecular masses of these proteins were surprisingly low. Clark et al. (14) indicated that the low molecular mass might mean an unexpectedly short *recE* gene. Chu et al. (10) supported this by discussing the possibility that wild-type RecE nuclease might have abnormally low mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) leading to overestimates of molecular mass. The short-gene hypothesis was one of two hypotheses put forward by Luisi-DeLuca et al. (29). The alternative was that there was processing of the mutant RecE proteins encoded by the deletion mutant genes. In this paper we investigate the nucleotide sequence of the C-terminal portion of *recE* to clarify this situation.

A study of two other external deletions affecting *recE* expression has also been published (30). One of the two external deletions had fused the N-terminal portion of a gene called *sfcA* to the C-terminal portion of *recE*. In this case a fusion protein of 150 kDa was visible, indicating that the alteration of mobility in SDS-PAGE produced in the case of the *racC-recE* fusions did not occur. This difference between *sfcA* and *racC* external deletions must also be explained.

MATERIALS AND METHODS

Plasmids and oligonucleotides used for DNA sequencing and PCR procedures. A series of deletion mutant plasmids was used to determine the nucleotide sequence of the C terminus of *recE* and to analyze the function of this region. pRAC1 was the starting plasmid (Fig. 1A). A deletion of 2,041 nucleotides produced pRAC7 (10, 40). pRAC7 DNA was treated with *Cla*I nuclease and ligase to produce pJC980 (Fig. 1A). Controlled digestion by DNA exonuclease III (ExoIII) of pJC980 DNA, which had been cleaved by *Bst*EII and *Cla*I nucleases, was used to produce a series of further deletions (Fig. 1B and see

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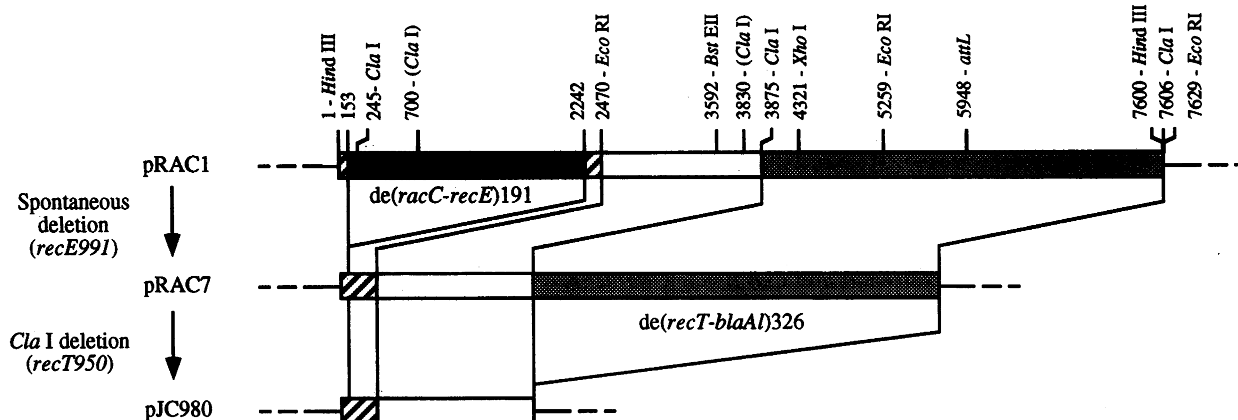

FIG. 1. Diagram of the deletion mutations used to analyze the C-terminal region of the *recE* gene. (A) Two steps of deletion were used to construct pJC980 from the plasmid pRAC1 (40). The large rectangle represents the 7.6-kb *Hin*dIII fragment cloned from *E. coli* K-12 strain KL16. Lines at each end represent portions of the vector pBR322. Numbers on the large rectangle refer to nucleotide coordinates beginning at a *Hin*dIII cleavage site (10). Shaded portions of the large rectangle represent those regions deleted from pRAC1 to make pJC980. Two *Cla*I cleavage sites are protected from cleavage by methylation of overlapping GATC sequences. These are indicated by parentheses. In the first step a spontaneous deletion, called del(*racC-recE*)*191* by Willis et al. (40), converted pRAC1 to pRAC7. The endpoints of the deletion del(*racC-recE*)*191* were determined by Chu et al. (10), who published the sequence represented by the striped and striped-shaded rectangles. pRAC7 was used to make pJC980 because del(*racC-recE*)*191* had deleted the *Cla*I site at 245. In the second step pRAC7 DNA was treated with *Cla*I endonuclease and ligase as described in the text. One endpoint of the deletion was in the leader sequence transcribed from the *bla-p2* promoter of pBR322. The other was found to be located in an open reading frame (*orf*), which we call *recT* (see Results). The name of the deletion [del(*blaAl-recT*] represents these endpoints by the convention of Demerec et al. (17). We also call the *recT* allele *recT950*. (B) Diagram of the deletions initiated at the *Cla*I and *Bst*EII sites in pJC980 as described in the text. Heavily shaded rectangles represent the DNA deleted. The left endpoints in the cloned DNA are numbered according to the convention mentioned in the legend to panel A. The right endpoints lie in the vector and are numbered according to the convention for pBR322 (38). The dashed lines represent the estimates for the boundaries between domains 2 and 3 of the *recE* gene described by Luisi-DeLuca et al. (29). From the sequence each deletion allele except *recT951* is expected to encode a mutant protein with the following number of amino acids (aa) (calculated molecular mass in parentheses): RecT958 protein, 358 aa (39.3 kDa); RecT959 protein, 340 aa (37.3 kDa); RecET960 protein, 276 aa (31.3 kDa); RecET962 protein, 254 aa (28.9 kDa); and RecET963 protein, 296 aa (33.8 kDa). RecT958 and RecT959 proteins are expected to have 73 and 55 aa of RecT protein, respectively, fused to 285 aa of β-lactamase. pJC1501 was made without treatment by ExoIII and is expected to have the filled-in *Bst*EII and *Cla*I termini fused. The deletion was not sequenced to confirm expectation.

below). The oligonucleotide primers used for DNA sequencing and polymerase chain reaction (PCR) procedures are listed in Table 1.

ExoIII treatment to produce deletions. The procedure of Henikoff (22), modified slightly, and reagents from Promega Co. were used. pJC980 DNA was purified by CsCl-ethidium bromide gradient centrifugation. Ten micrograms of this DNA was treated with *Cla*I nuclease and then extracted with phenol-chloroform and precipitated with ethanol. The DNA was then incubated with the Klenow fragment of DNA polymerase I (*Pol*I) and alpha-phosphorothioate nucleotides and again extracted and precipitated. Next, the DNA was treated with *Bst*EII nuclease before extraction and precipitation. The DNA was then incubated at 32°C with 540 U of ExoIII. Every 20 s, samples were removed. ExoIII digestion was inhibited by adding the samples to a chilled solution of S1 nuclease, and

TABLE 1. Oligonucleotide primers used for sequencing and for locating the Tn*10* in *rec-101*::Tn*10*

Name	Sequence location	Sequence
Top strand[a]		
prJC9	2176-2193[b]	5′ GGCGCTGAACATCCGCAC
prJC58	2353-2370[b]	5′ GACATTGCTGATACTCCG
prJC59	2755-2771	5′ TCACTGGATCATGGACG
prJC39	2872-2888	5′ TGGAGTGCAGCCAACTT
Bottom strand[c]		
prJC57	2595-2578	5′ TCTTCCGCAGTGATAACC
prJC47	3256-3240	5′ CGGATCATACGTTCAGC
prJC60	3927-3908	5′ AGTTGGCGGTGCATTACACC
prJC61	3499-3480	5′ GACAGGCTGGCGATTTGACC
prJC62	3766-3747	5′ TTGAACAGGCGACGAATAGC

[a] Identical to the sequence in Fig. 2.
[b] Refers to the sequence of Chu et al. (10).
[c] Complementary to the sequence in Fig. 2.

this mixture was then incubated for 30 min at room temperature. (In the Henikoff procedure, ExoIII was heat inactivated and the ExoIII-treated DNA was isolated before exposure to S1 nuclease.) Reactions were stopped by addition of 0.5 M Tris, pH 8, and 0.125 M EDTA and incubation at 70°C for 10 min. Finally the DNA was treated first with the Klenow fragment of DNA polymerase I and a deoxynucleoside triphosphate (dNTP) mixture and then with T4 DNA ligase before it was used to transform JC5519. Ampicillin-resistant colonies were selected and screened by the mini-DNA-prep method of Birnboim and Doly (4) and agarose gel electrophoresis to select those with increasingly longer deletions.

Sequencing strategy. A series of M13 mp8 and mp9 clones were made to carry portions of an *Eco*RI-to-*Xho*I fragment of pRAC7. They were called JCM phage strains and are listed in Table 2. These yielded nucleotide sequence information for approximately 89% of one strand and approximately 72% of the other strand. To fill in the missing sequence we used three strategies. The missing sequence for one strand was provided from plasmid pSJS251 by using primers prJC39 and prJC59. Some of the missing sequence from the complementary strand was obtained by cloning fragments from deletion mutant derivatives of pRAC7 (see below). The rest of the missing sequence was obtained by cloning the *Eco*RI-to-*Hin*dIII segment from pRAC3. To show that only one *Eco*RI cleavage sequence was present in *recE*, we used prJC58 and pRAC1 DNA.

The sequence as originally read contained an open reading frame of 1,425 nucleotides continuous with the *recE* open reading frame of Chu et al. (10). A *Bgl*I cleavage sequence was present from nucleotides 591 to 601 in this reading frame. An attempt to cleave this sequence for subcloning the downstream region revealed that cleavage did not occur. When we reread the original sequence gels, we discovered an extra G between positions 598 and 599 which eliminated the *Bgl*I sequence. Because addition of the G also prematurely terminated the continuous *recE* open reading frame, we redid the sequence of both top and bottom strands from three more plasmids: pSJS251, pRAC26, and pRAC31. In each case the extra G between 598 and 599 was seen. All three plasmids had been derived from pRAC1. pRAC26 and pRAC31 were *recE*$^+$ (40) and produced 140-kDa RecE proteins (28). pSJS251 is described below. Truncation of the *recE* reading frame created a new reading frame which we call *recT*. In this paper we show that *recT* is necessary for conjugational recombination. Hall et al. (20) describe the RecT protein.

Nucleotide sequencing procedures. Single-strand DNA cloned in M13 strains was sequenced by the chain termination method of Sanger et al. (35). Supercoiled plasmid DNA was sequenced by the method of Chen and Seeberg (9).

***recE* and *recT* plasmids.** The step which revealed the absence of the *Bgl*I recognition sequence was the cloning of *recT* downstream of the *lacZ* promoter and operator (*lacZp* and *lacZo*, respectively) in the plasmid vector pMC9. This plasmid contains a 1,724-nucleotide *Hin*dII partial-digest fragment carrying *lacIp lacI lacZo lacZp* and the first 160 or so codons of *lacZ* (7). This fragment was inserted via linkers into *Eco*RI-cleaved pBR322 (6). We replaced a 628-bp *Cla*I-*Sal*I fragment of pMC9 with a 1,685-bp *Bgl*I-*Xho*I fragment from pRAC1 containing the C-terminal 148 codons of *recE* (*recE944*), all of *recT*, and two other open reading frames (see Results). By treating *Cla*I-cut pMC9 DNA and *Bgl*I-cut pRAC1 DNA with *Pol*I Klenow fragment in the presence of dNTPs, the ends were blunted. Because of the complementarity of *Sal*I and *Xho*I termini, *recT* was cloned in such a way that its transcription was regulated by *lacZo lacZp*. The resulting 7.1-kb plasmid,

TABLE 2. M13 phage strains used for DNA nucleotide sequencing

Phage strain JCM numbers	Phage vector	Cloned fragment	Source plasmid	Original pRAC plasmid	Reference for source
14679	mp8	*Cla*I-*Cla*I	pRAC36	pRAC36	40
15918	mp8	*Eco*RI-*Eco*RI	pSKM1	NA[a]	30
16232	mp8	*Pst*I-*Xho*I	pJC912	pRAC7	This work
16234	mp9	*Pst*I-*Xho*I	pJC912	pRAC7	This work
16260	mp18	*Nsi*I-*Hin*cII	pJC911	pRAC7	This work
16262	mp19	*Nsi*I-*Pst*I	pJC911	pRAC7	This work
16264	mp19	*Hin*cII-*Nsi*I	pJC911	pRAC7	This work
17306	mp8	*Eco*RI-*Eco*RI	pJC1506	pRAC7	This work
17308	mp8	*Eco*RI-*Xmn*I	pJC1508	pRAC7	This work
17309	mp8	*Eco*RI-*Xmn*I	pJC1509	pRAC7	This work
17310	mp8	*Eco*RI-*Ssp*I	pJC1510	pRAC7	This work
17311	mp8	*Eco*RI-*Ssp*I	pJC1511	pRAC7	This work
17312	mp8	*Eco*RI-*Ssp*I	pJC15123	pRAC7	This work
17313	mp8	*Eco*RI-*Ssp*I	pJC1513	pRAC7	This work
17314	mp9	*Hin*dIII-*Ssp*I	pJC1514	pRAC7	This work
17321	mp9	*Hin*dIII-*Eco*RI	pRAC3	pRAC3	40

[a] NA, not applicable.

TABLE 3. *recE* and *recT* plasmids

Group and plasmids	Genotype	Repression system	Vector	Source of *recE* and *recT*
Group A: IPTG-derepressible *recT* plasmids				
pSJS251	*recE944 recT*⁺	*lacI lacZo*	pMC9	pRAC1
pJC1549	*recE944 recT942*::KIXX	*lacI lacZo*	pSJS251	pSJS251
Group B: 42C-derepressible *recT* plasmids				
pJC1548	*recE944 recT*⁺	*cIAt2 oLoR*	pSJS126	pSJS251
pJC1553	*recE944 recT*⁺	*cIAt2 oLoR*	pJC1551	pJC1548
pJC1554	*recE944 recT943*::KIXX	*cIAt2 oLoR*	pJC1553	pJC1553
Group C: pBR322-compatible *recT* plasmid (pJC1572)	*recE944 recT*⁺	*lacI lacZo*	pACYC184	pSJS251
Group D: High-copy-number *recE* and *recT* plasmids				
pJC1544	*recE991 recT950*	*lacI lacZo*	pUC19	pJC980
pJC1546	*recE960 recE991 recT960*	*lacI lacZo*	pUC19	pJC1510
pJC1547	*recE944 recT945*	*lacI lacZo*	pUC18	pJSJ251
pJC1557	*recE991 recT959*	*lacI lacZo*	pUC19	pJC1509

pSJS251, was used to make a *recT* insertion mutant derivative (pJC1549) by cleaving pSJS251 DNA with *Sma*I and inserting a 1.2-kb *Sma*I fragment from pUC4-KIXX (Pharmacia). The inserted fragment carries *aphA* conferring kanamycin resistance. The mutation is called *recT942*::KIXX. Both pSJS251 and pJC1549 are called group A plasmids in Table 3.

A second group of plasmids (group B in Table 3) was made from pSJS251 to put *recT* under control of *cIAt2*, a temperature-sensitive repressor allele, and *pLoL pRoR* of lambda. pJC1548 is the first in this group and is derived from the vector pSJS126, a *recF*-carrying derivative of pUC118 (34). A 0.6-kb *Apa*I-*Eag*I fragment, containing the N terminus of *recF*, was removed from pSJS126. In its place was added a 1.9-kb *Eag*I-*Eco*RI fragment carrying *recT* flanked by portions of pBR322. Directional cloning was ensured by blunting the *Apa*I and *Eco*RI termini with *Pol*I Klenow fragment. pJC1548 contains two *Sma*I cleavage sequences, however, so it was not possible to make an *aphA* (Km^r) insertion mutant. To remedy this, we recloned *recT* from pJC1548 by using the modified pUC118 vector pJC1551. To make pJC1551, pUC118 DNA was cleaved with *Sma*I and the resulting single-stranded termini were filled in by treatment with *Pol*I Klenow fragment and dNTPs. Ligation circularized the plasmid and destroyed the *Sma*I cleavage sequence. *Sac*I *Eag*I digestion of pJC1548 removed *recT* and the controlling *pLoL pRoR cIAt2* sequences on a 2.9-kb fragment. By blunting the *Eag*I terminus of the fragment, *recT* was inserted directionally into *Eco*RI-*Sac*I-treated pJC1551 DNA which had the *Eco*RI terminus filled in. DNA from the resulting *recT*⁺ plasmid, pJC1553, was treated with *Sma*I, and the 1.2-kb *Sma*I fragment carrying *aphA*⁺ (Km^r) from pUC4-KIXX was inserted. The resulting *recT943*::KIXX plasmid was called pJC1554.

Another plasmid (the only member of group C in Table 3) was made from pSJS251 in order to have a plasmid compatible with pBR322-derived and pUC-derived plasmids. This plasmid, pJC1572, is compatible because it is derived from pACYC184 (8). *Eag*I *Eco*RV nuclease digestion of pACYC184 DNA removes a 0.7-kb fragment of the *tetA* gene. This was replaced with a 3.7-kb *Eag*I partial *Eco*RI-digested fragment from pSJS251 to create a 7.2-kb plasmid. The partial *Eco*RI digest was done to prevent separation of *recT* from the *lac* control genes.

Finally, a group of three pUC19-derived plasmids and one pUC18-derived plasmid (group D in Table 3) was constructed to raise the copy number of deletion mutant forms of the *recE* and *recT* genes. To make the pUC19 derivatives, *Hin*dIII-*Xmn*I fragments were obtained from pJC980, pJC1509, and pJC1510. These fragments were 2.3, 1.4, and 1.3 kb, respectively. They were inserted into *Hin*dIII-*Sma*I-digested pUC19 DNA by ligation. The resulting plasmids are pJC1544, pJC1557, and pJC1546, respectively. The pUC18 derivative was made by *Eco*RI *Cla*I cleavage of pSJS251, which removed a 1.2-kb fragment containing the C-terminal 207 codons of *recE* and 264 of the 269 codons of *recT*. This was added to pUC18 DNA cleaved with *Eco*RI and *Ace*I nucleases. The resulting plasmid, pJC1547, encodes a mutant but functional RecT protein in which the carboxy-terminal 4 amino acids have been substituted with 15 amino acids of the *lacZ* portion of pUC18. In all these plasmids the *recE* and *recT* alleles are under *lacI lacZo lacZp* control.

Genetic procedures. Conjugation was carried out essentially as described by Clark (12) to obtain the results in Table 4. To obtain the results in Table 5, however, modifications were made. For example, 2-ml rather than 5-ml mating mixtures were used, incubation was in 16-ml centrifuge tubes rather than 125-ml Erlenmeyer flasks, and the mixtures were aerated by gentle shaking rather than incubated motionless. Media were described by Adelberg and Burns (1). Recipient cultures were grown in L medium plus antibiotics. Since the Hfr strains used were sensitive, the antibiotics were removed by centrifugation and the recipient cells were resuspended in antibiotic-free L medium prior to addition of Hfr cells. After 60 min of incubation, cultures were centrifuged to remove L medium. Mating aggregates were resuspended at the original density in 56/2 salts buffer. Aggregates were then disrupted by shaking in a vortex mixer for 40 s.

Transformation was performed as described by Brown et al. (5). Tests for UV and mitomycin sensitivity were performed as described by Clark and Margulies (13) and Kushner et al. (26), respectively.

Western immunoblots. Proteins were visualized by enhanced chemiluminescence after they were separated by electrophoresis and treated with polyclonal antiserum made against ExoVIII protein purified by the method of Luisi-DeLuca et al. (28). We used an ECL kit from Amersham Corporation and the directions contained therein.

Location of Tn*10* in *rec-101*::Tn*10*. Tn*10* in *rec-101*::Tn*10* was located roughly by a Southern blot method (14, 39). DNA oligonucleotides bracketing this expected position were synthesized and used as primers to verify this location by the PCR

TABLE 4. Phenotypes of strains[a] carrying deletion mutant derivatives of pRAC7

Plasmid	Genotype	U of nuclease[b]	% Recombinants[c] TL$^+$ (Ser$^+$ Sm^r Amp^r)	% Survival	
				UV[d]	Mitomycin[e]
pRAC1	*recE939 recT*$^+$	ND[f]	0.0067	0.0078	0.068
pRAC7	*recE991 recT*$^+$	ND	17	26	71
pJC980	*recE991 recT950*	ND	3.3	20	39
pJC1501	*recE991 recT951*	93	0.11	1.5	0.45
pJC1506	*recE991 recT956*	98	0.13	3.4	0.63
pJC1508	*recE991 recT958*	ND	ND	1.1	0.56
pJC1509	*recE991 recT959*	250	0.099	2.0	0.88
pJC1510	*recE991 recE960 rec960*	<5	0.075	0.029	0.036
pJC1512	*recE991 recE962 recT962*	ND	ND	0.019	0.053
pJC1513	*recE991 recE962 recT962*	ND	ND	0.019	0.060
pJC1514	*recE991 recE964 recT964*	<5	0.099	0.035	0.048

[a] Host strain is JC5519 (*recB21 recC22* Rac$^-$).
[b] Nuclease assays were performed as described by Luisi-DeLuca et al. (29).
[c] One hour mating interrupted by vortexing. Hfr was JC11033, an HfrH derivative.
[d] 40 J/m^2.
[e] 1.0 μg/ml.
[f] ND, not determined.

method of Mullis and Faloona (31). prJC39 was used to synthesize one strand, and four other oligonucleotides (prJC47, prJC61, prJC62, and prJC60) at increasing distances from prJC39 were used one at a time to synthesize the other strand. From wild-type DNA the pairwise combinations produced fragments of 387, 628, 894, and 1,054 bp by using the following cycling protocol: 5 min at 94°C; 40 cycles of 2 min at 94°C, 2 min at 55°C, and 2 min at 72°C; and finally 5 min at 72°C and storage at 4°C until analyzed. From *rec-101*::Tn*10* DNA, only prJC47 and prJC61 produced fragments with prJC39, presumably because Tn*10* consisting of 9.3 kb of DNA (21) is inserted between the positions of prJC61 and prJC62 and the polymerizing cycle time is inadequate to complete fragment synthesis.

Nucleotide sequence deposition. The nucleotide sequence of the *Eco*RI (2470)-to-*Xho*I (4327) fragment of pRAC1 (Fig. 1) has been deposited in GenBank, accession number L23927.

RESULTS

Sequencing analysis of the *Eco*RI-to-*Xho*I fragment of pRAC1. Figure 2 shows the nucleotide sequence of the *Eco*RI-to-*Xho*I fragment originally cloned as part of pRAC1 (40).

TABLE 5. *trans* complementation of *recE* and *recT*

Plasmid[a]	% Recombination[b]		% Survival (40 J of UV per m^2)	
	No *recT* (pACYC184)	*recT*$^+$ (pJC1572)	No *recT* (pACYC184)	*recT*$^+$ (pJC1572)
None	ND[c]	0.039	0.31	0.20
pBR322	0.012	0.027	0.023	0.038
pJC980	0.90	21.5	25	44
pJC1509	0.070	12.8	7.1	32
pJC1510	0.073	0.15	0.17	1.3

[a] All plasmids are present in the JC5519 background. The *recB*$^+$ *recC*$^+$ strain AB1157, by contrast, gave 89% recombination.
[b] Crosses were performed with Hfr strain JC158 (PO1). To induce transcription of *recT*, IPTG at a final concentration of 0.5 mM was added to all recipient cultures at the same time as addition of Hfr cells. For 60 min the mixtures of Hfr and F$^-$ cells were incubated at 37°C in complex (L) medium without antibiotics. Selection was made for TL$^+$ (Ser$^+$ Sm^r Cm^r Amp^r) recombinants or TL$^+$ (Ser$^+$ Sm^r Cm^r) recombinants in one case.
[c] ND, not determined.

Four long open reading frames are seen by translating the strand shown in the figure. All four have been analyzed by the program CODONUSE and been found to have high odds of being translated in vivo (data not shown). One of these is a continuation of the *recE* gene whose N-terminal 661 codons were previously published (10). The first two codons in Fig. 2 correspond to the *Eco*RI recognition sequence and are included in the 661. The C-terminal portion of *recE* consists of 205 translated codons. The full-sized gene, therefore, is predicted to encode a protein of 866 amino acids and a calculated molecular mass of 96.2 kDa. Partially overlapping *recE* is an open reading frame we call *recT* because deletion analysis indicates its necessity for conjugational recombination (see below). There are three potential translational start signals (i.e., ATG codons) near the terminus of *recE*. We have chosen the third of these because the predicted sequence following it corresponds to the N-terminal amino acid sequence of purified RecT protein (20). *recT* is, therefore, predicted to encode a protein of 269 amino acids with a calculated molecular mass of 29.7 kDa.

Separated from *recT* by 17 nucleotides is an open reading frame (*orfG*) for which we have no evidence of expression. Were it translated, its protein would be 77 amino acids with a calculated molecular mass of 8.7 kDa. Partially overlapping *orfG* is *orfH* which terminates 24 nucleotides into the adjacent fragment (36). There are three potential ATG translation start codons near the terminus of *orfG*. If the third of these is used, by analogy with *recT*, an OrfH protein is predicted to consist of 69 amino acids and have a molecular mass of 7.8 kDa.

By translating the strand complementary to that shown in Fig. 2, 10 complete open reading frames, which vary in size from 39 to 138 codons between potential ATG initiation and nonsense termination codons, can be seen. According to the program CODONUSE, one of these shows high odds of in vivo translation and three each are in qualitative categories of no, low, and moderate odds of in vivo translation. Preliminary evidence indicates that one of the low-odds *orfs* may be expressed as the *ral* gene of Rac (36).

Computer analysis. Using the program GENALIGN the C-terminal 281 amino acids encoded by *recE* were compared to the 226 amino acids of the Lambda RedX exonuclease. Except for one group of four residues (VAPE), only scattered amino acid identities were noted by making 27 gaps in the RecX

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          E  F  S  N  R  F  I  V  A  P  E  F  N  R  R  T  N  A  G  K  E  E  E  K  A  F  L  M  E  C  A  T  A  G  K   RecE
1(2470)   GAATTCAGTAACCGCTTTATCGTAGCACCTGAATTTAACCGCCGTACAAACGCCGGAAAAGAAGAAGAGAAAGCGTTTCTGATGGAATGCGCAACAGCAGGAAAA
          EcoRI

          T  V  I  T  A  E  E  G  R  K  I  E  L  M  Y  Q  S  V  M  A  L  P  L  G  Q  W  L  V  E  S  A  G  H  A  E   RecE
106(2575) ACGGTTATCACTGCGGAAGAAGGCCGGAAAATTGAACTCATGTATCAAAGCGTTATGGCTTTGCCGCTGGGGCAATGGCTTGTTGAAAGCGCCGGACACGCTGAA
                                                                           BglI

          S  S  I  Y  W  E  D  P  E  T  G  I  L  C  R  C  R  P  D  K  I  I  P  E  F  H  W  I  M  D  V  K  T  T  A   RecE
211(2680) TCATCAATTTACTGGGAAGATCCTGAAACAGGAATTTTGTGTCGGTGCCGTCCGGACAAAATTATCCCTGAATTTCACTGGATCATGGACGTGAAAACTACGGCG

          D  I  Q  R  F  K  T  A  Y  Y  D  Y  R  Y  H  V  Q  D  A  F  Y  S  D  G  Y  E  A  Q  F  G  V  Q  P  T  F   RecE
316(2785) GATATTCAACGATTCAAAACCGCTTATTACGACTACCGCTATCACGTTCAGGATGCATTCTACAGTGACGGTTATGAAGCACAGTTTGGAGTGCAGCCAACTTTC
                                                1513                        1512

          V  F  L  V  A  S  T  T  I  E  C  G  R  Y  P  V  E  I  F  M  M  G  E  E  A  K  L  A  G  Q  Q  E  Y  H  R   RecE
421(2890) GTTTTTCTGGTTGCCAGCACAACTATTGAATGCGGACGTTATCCGGTTGAAATTTTCATGATGGGCGAAGAAGCAAAACTGGCAGGTCAACAGGAATATCACCGC
                                                                                                           1510

          N  L  R  T  L  S  D  C  L  N  T  D  E  W  P  A  I  K  T  L  S  L  P  R  W  A  K  E  Y  A  N  D  Z       RecE
                                                                                                   M  T  K  Q  P  RecT
526(3055) AATCTGCGAACCCTGTCTGACTGCCTGAATACCGATGAATGGCCAGCTATTAAGACATTATCACTGCCCCGCTGGGCTAAGGAATATGCAAATGACTAAGCAACCA

          P  I  A  K  A  D  L  Q  K  T  Q  G  N  R  A  P  A  A  V  K  N  S  D  V  I  S  F  I  N  Q  P  S  M  K  E   RecT
632(3101) CCAATCGCAAAAGCCGATCTGCAAAAAACTCAGGGAAACCGTGCACCAGCAGCAGTTAAAAATAGCGACGTGATTAGTTTTATTAACCAGCCATCAATGAAAGAG

          Q  L  A  A  A  L  P  R  H  M  T  A  E  R  M  I  R  I  A  T  T  E  I  R  K  V  P  A  L  G  N  C  D  T  M   RecT
737(3206) CAACTGGCAGCAGCTCTTCCACGCCATATGACGGCTGAACGTATGATCCGTATCGCCACCACAGAAATTCGTAAAGTTCCGGCGTTAGGAAACTGTGACACTATG
                                                 1509                                                   1508

          S  F  V  S  A  I  V  Q  C  S  Q  L  G  L  E  P  G  S  A  L  G  H  A  Y  L  L  P  F  G  N  K  N  E  K  S   RecT
842(3311) AGTTTTGTCAGTGCGATCGTACAGTGTTCACAGCTCGGACTTGAGCCAGGTAGCGCCCTCGGTCATGCATATTTACTGCCTTTTGGTAATAAAAACGAAAAGAGC

          G  K  K  N  V  Q  L  I  I  G  Y  R  G  M  I  D  L  A  R  R  S  G  Q  I  A  S  L  S  A  R  V  V  R  E  G   RecT
947(3416) GGTAAAAAGAACGTTCAGCTAATCATTGGCTATCGCGGCATGATTGATCTGGCTCGCCGTTCTGGTCAAATCGCCAGCCTGTCAGCCCGTGTTGTCCGTGAAGGT

           D  E  F  S  F  E  F  G  L  D  E  K  L  I  H  R  P  G  E  N  E  D  A  P  V  T  H  V  Y  A  V  A  R  L  K  RecT
1052(3521) GACGAGTTTAGCTTCGAATTTGGCCTTGATGAAAAGTTAATACACCGCCCGGGAGAAAACGAAGATGCCCCGGTTACCCACGTCTATGCTGTCGCAAGACTGAAA
                                                            SmaI                 BstEII
                                                                                  1501

           D  G  G  T  Q  F  E  V  M  T  R  K  Q  I  E  L  V  R  S  L  S  K  A  G  N  N  G  P  W  V  T  H  W  E  E  RecT
1157(3626) GACGGAGGTACTCAGTTTGAAGTTATGACGCGCAAACAGATTGAGCTGGTGCGCAGCCTGAGTAAAGCTGGTAATAACGGGCCGTGGGTAACTCACTGGGAAGAA

           M  A  K  K  T  A  I  R  R  L  F  K  Y  L  P  V  S  I  E  I  Q  R  A  V  S  M  D  E  K  E  P  L  T  I  D  RecT
1262(3731) ATGGCAAAGAAAACGGCTATTCGTCGCCTGTTCAAATATTTGCCCGTATCAATTGAGATCCAGCGTGCAGTATCAATGGATGAAAAGGAACCACTGACAATCGAT

           P  A  D  S  S  V  L  T  G  E  Y  S  V  I  D  N  S  E  E  Z                    M  H  R  Q  L  E  I  F  F  OrfG
1367(3836) CCTGCAGATTCCTCTGTATTAACCGGGGAATACAGTGTAATCGATAATTCAGAGGAATAATTCAGCCTGGCGGTGTAATGCACCGCCAACTTGAAATATTTTTT
            PstI                                    ClaI

           M  R  K  I  M  R  Y  D  N  V  K  P  C  P  F  C  G  C  P  S  V  T  V  K  A  I  S  G  Y  Y  R  A  K  C  N  OrfG
1471(3940) ATGAGAAAAATTATGAGATATGACAATGTTAAACCATGTCCATTTTGTGGTTGTCCATCAGTAACGGTGAAAGCCATTTCAGGATATTACCGAGCGAAGTGTAAC

           G  C  E  S  R  T  G  Y  G  G  S  E  K  E  A  L  E  R  W  N  K  R  T  T  G  N  N  N  G  G  V  H  V  Z     OrfG
                                                                                                   M  Y  K     OrfH
1576(4045) GGATGCGAATCCCGAACCGGTTATGGTGGAAGTGAAAAAGAAGCACTCGAAAGATGGAATAAACGAACCACTGGAAATAATAATGGAGGTGTTCATGTATAAA

           I  T  A  T  I  E  K  E  G  G  T  P  T  N  W  T  R  Y  S  K  S  K  L  T  K  S  E  C  E  K  M  L  S  G  K  OrfH
1679(4148) ATTACCGCCACTATTGAAAAGGAAGGTGGCACTCCTACTAACTGGACAAGATATTCAAAATCTAAACTAACGAAATCAGAATGCGAAAAAATGCTCTCAGGTAAA

           K  E  A  G  V  S  R  E  Q  K  V  K  L  I  N  F  N  C  E  K  L  Q  S  S    OrfH
1784(4253) AAAGAAGCAGGCGTTTCCAGAGAGCAGAAAGTAAAACTGATAAATTTTAATTGCGAGAAACTTCAGTCCTCGAG
                                                                              XhoI
```

sequence. The same program was used to compare the RecT and RedB (beta) proteins of lambda. Here, too, scattered identities were seen but because the two proteins differ in length by only 8 amino acids, only four gaps were necessary to make the optimal alignment.

The nucleotide sequence of lambda was scanned for similarities to *recE* and *recT*. To do this we had to compensate for the fact that the lambda *redX* and *redB* genes are transcribed from left to right as the standard map of lambda is drawn (15). Hence the lambda sequence as usually written, 5′ to 3′ (16), contains the complement of *redX* and *redB*. Thus we compared the complement of the sequence shown in Fig. 2 with the lambda sequence. A 44.2% identity was found between 2,475 nucleotides of the *recE* complement and 2,484 nucleotides of lambda. This correspondence involved the complement of codons 17 to 842 of *recE* with nucleotides 25876 to 23392 of lambda. According to the map of lambda (15, 16) this region includes the C-terminal 160 codons of EA59, all of EA31, and the N-terminal 175 codons of EA47. All three of these lambda genes are transcribed in the same direction as the *red* genes. The genes are in the b2 region, and EA59 encodes a DNA endonuclease. Alignment of the amino acid sequences of these three lambda proteins with the appropriate portions of RecE protein revealed scattered amino acid identities.

Deletion analysis of the *EcoRI*-to-*XhoI* fragment. A series of deletion mutant derivatives of pRAC7 were produced in order to determine the biological activities of *recT* and the C terminus of *recE*. pRAC7 was used rather than pRAC3, whose nuclease product has been purified (20, 29), because a *Cla*I nuclease recognition sequence was deleted in pRAC7 and not in pRAC3. The first deletion derivative was pJC980 (Fig. 1A and B). In this derivative the last four translated codons of *recT* have been removed (see the *Cla*I sequence in Fig. 2). In their place are 15 translated codons from pBR322. This substitution, which we call *recT950*, has little effect on the phenotype of *recB recC* double mutant JC5519 (Table 4). Recombinant frequency decreases fivefold, and survival with UV irradiation and mitomycin treatment is slightly reduced. The next deletion, to make pJC1501, removes about 40% of *recT* (Fig. 1B and Fig. 3) and has a severe effect on phenotype, although it does not inactivate the nuclease activity associated with the mutant form of ExoVIII encoded by pRAC7 (Table 4). Additional deletions to make pJC1508 and pJC1509 do not alter the phenotype much more than the deletion in pJC1506 (Table 4), although they remove increasing amounts of *recT* (Fig. 1B). The first deletion to remove part of *recE* is in pJC1510 (Fig. 1B); it inactivates the nuclease activity and reduces survival with both UV and mitomycin treatment (Table 4). Recombinant frequency, however, is not further reduced (Table 4). This analysis, therefore, reveals that *recT* is required for conjugational recombination and complete recovery from UV and mitomycin damage. This analysis is mute about the requirement for the nuclease-encoding region of *recE* in recombination, although it does indicate that some recovery from UV and mitomycin damage depends on the nuclease.

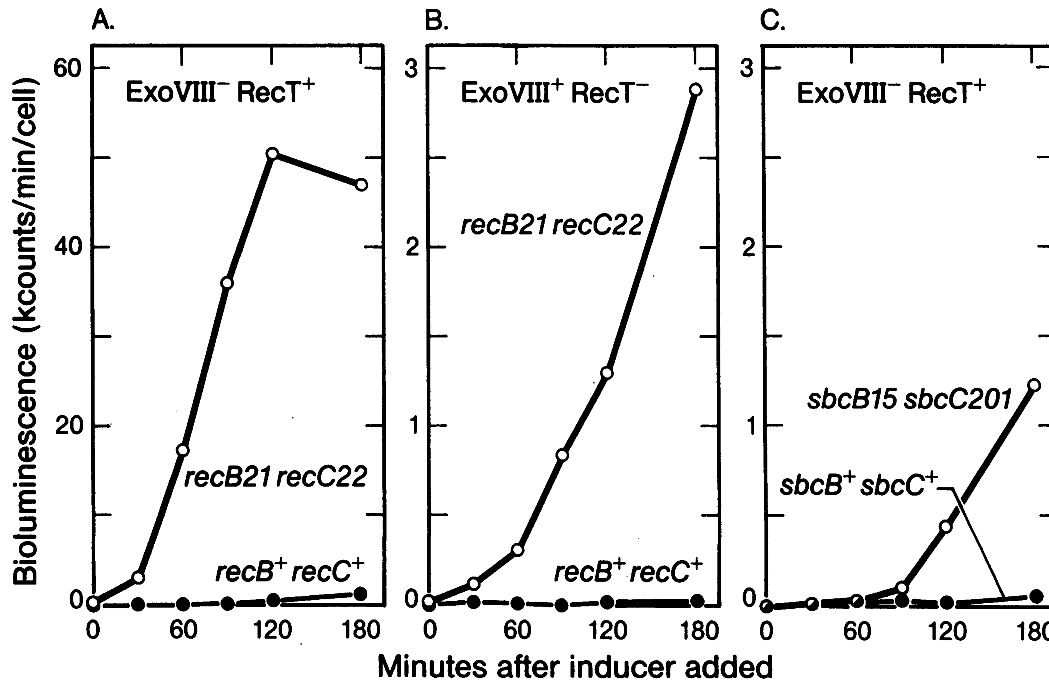
Requirement for RecE991 nuclease in genetic recombination. To test the need for RecE nuclease in genetic recombination, it is necessary to have *recE* mutants which are *recT*$^+$. We have reason to believe that one mutant which was called a *recE* mutant in the past, *recE101*::Tn*10* (18), is actually a *recT* mutant (see below). Another mutation, *recE159*, results in a truncated ExoVIII protein which migrated in SDS-PAGE as if it had a molecular mass of 106 kDa (28). Since a protein chain-terminating mutation might have a polar effect on *recT*, it might make cells effectively *recT* as well as *recE* mutants. Thus, previously published results contain no clear-cut genetic evidence that *recE* nuclease activity is required for recombination.

To remedy this situation, we have used *recE* and *recT* genes carried by compatible plasmids in a *cis-trans* test. The *recE* gene used is the deletion mutant form *recE991* found in pRAC7. Table 5 shows that *recT*$^+$ alone in pJC1572 will not support conjugational recombination with or without pBR322 as a coresident plasmid. When *recT950* is located *cis* with *recE991* in pJC980, about 1% recombination occurs. When *recT*$^+$ on pJC1572 is located *trans* to *recE991* on pJC1509, about 13% recombination occurs. Deletion of the C terminus of *recE991*(pJC1510) reduces recombination about 100-fold in the presence of *recT*$^+$(pJC1572). Thus both *recE* and *recT* activities are required for conjugational recombination in the *recB21 recC22 sbcB*$^+$ genetic background used.

***recT* can substitute for *redB* in plasmid recombination.** Lambda phage carries a nuclease gene (*redX*) whose product (Lambda Exo) is required for lambda recombination (33, 37) and is isofunctional with the RecE nuclease (19, 23, 27). Lambda also carries another recombination gene (*redB*) contiguous to *redX* (33, 37). The product of *redB*, called beta protein, catalyzes renaturation of denatured DNA (24) and is also required for lambda recombination. Using the bioluminescence reporter gene system of Nussbaum and Cohen (32), Berger and Cohen (3) found that *redB* stimulates *recA*-independent recombination of bacterial plasmids. To test the in vivo activity of *recT*, we repeated their experiments substituting *recT* for *redB*. Panel A in Fig. 3 shows that substantial recombination can be seen 30 min after *recT* is induced by IPTG (isopropyl-β-D-thiogalactopyranoside). The amount of bioluminescence increases linearly over the next 90 min. Similar induction of the nuclease portion of *recE*, however, produces an average of only 3% as much recombination (panel B; note the difference in ordinate scales between panels A and B). These tests were done in the optimal genetic background for detecting *red*-dependent *recA*-independent recombination (3, 32), i.e., del(*recA*)*306 recB21 recC22 sbcB15 sbcC201*. Further tests showed that the *recB*$^+$ *recC*$^+$ genes inhibited RecT-dependent recombination (Fig. 3A) and that the *sbcB*$^+$ *sbcC*$^+$ genes inhibited the residual RecT-dependent recombination in the *recB*$^+$ *recC*$^+$ strain (Fig. 3C). Similar effects were noticed on RedB-dependent recombination (3, 32).

Discrepancies between molecular mass estimates for ExoVIII and the length of *recE*. Clark et al. (14) pointed out a

FIG. 2. Nucleotide sequence of the *Eco*RI (2470)-to-*Xho*I (4327) fragment of pRAC1 (Fig. 1). Translation of the sequence reveals four long open reading frames. The *recE* reading frame begins in the adjacent *Hin*dIII-to-*Eco*RI fragment (10). *recT* and *orfG* are completely contained within this fragment. *orfH* begins in this fragment and terminates 21 nucleotides downstream (36). The translated products of the open reading frames are indicated in the right margin. Two numbering systems are indicated. One numbers nucleotides from the *Eco*RI sequence. The other (in parentheses) numbers from the *Hin*dIII sequence at the left of Fig. 1A (11). These correspond to the numbers in Fig. 1A. The endpoints of deletion mutations produced in pRAC7 are indicated by an underlined sequence and by a number below the underlined sequence, which is a pJC number indicating the plasmid carrying the mutation. The endpoint could not be uniquely determined in all cases because identical sequences in pBR322 and *recE* or *recT* had been used to make the junction. The extent of the sequence identity is indicated by underlining. A few relevant restriction enzyme cleavage sequences are indicated by underlining.



A. pSJS251 (*recE944 recT$^+$*) in del(*recA*)306 *sbcB15 sbcC201*
B. pJC1557 (recE991 recT959) in del(*recA*)306 *sbcB15 sbcC201*
C. pSJS251 (recE944 recT$^+$) in del(*recA*)306

FIG. 3. Recombination in *recA* mutant strains as measured by bioluminescence. Note that the ordinate scale of panel A is 20 times greater than the scales of panels B and C. Panels A and C show the effects of *recB recB sbcB sbcC* genotype on *recA*-independent intraplasmid recombination dependent on the *recT* gene. Panel B shows the effect of *recB recC* genotype on the small amount of *recA*- and *recT*-independent recombination dependent on the mutant *recE* gene of pRAC7.

discrepancy between the size of the *recE* gene predicted by molecular mass estimates of ExoVIII proteins and the estimated location of a Tn*10* insertion which appeared to eliminate ExoVIII activity and therefore had been called *recE101*::Tn*10* (39). This discrepancy was noted and further discussed by Luisi-DeLuca et al. (28), who showed that the insertion reduced but did not eliminate a protein whose mobility in SDS-PAGE was the same as that of wild-type ExoVIII protein. From the sequence presented here, we realized that the estimated location of *rec-101*::Tn*10* is in *recT*. To clear up this situation, we have used a PCR technique to locate the Tn*10* more accurately than the Southern blot method used previously (39). We find that it is located between 3499 and 3766 in *recT* and propose that it now be called *recT101*::Tn*10*.

Chu et al. (10) commented on the discrepancy between the 86-kDa molecular mass of frameshift mutant RecE939 protein estimated from SDS-PAGE and the 65-kDa mass predicted from the DNA sequence. They speculated that codons 257 to 355 of *recE* encode amino acids that might result in an abnormally low mobility of RecE939 protein in SDS-PAGE. Deletion of these codons by an essentially nested set of deletions, first described by Willis et al. (40), results in ExoVIII proteins whose estimated mass leads to an estimate of the location of the C terminus of *recE* (28) which corresponds closely with the location determined here from the nucleotide sequence (28). This differs from the location estimated from the mobility of another mutant protein, encoded by deletion *recE948*, which does not remove codons 257 to 355 and whose estimated C terminus matches that estimated from the mobility of wild-type ExoVIII protein (30).

The nucleotide sequence presented here leads to another suggestion for the discrepancy between the masses of ExoVIII protein estimated from the mobility on SDS-PAGE and calculated from the sequence of *recE*. ExoVIII protein may be a fusion product of RecE and RecT proteins because 125.9 kDa, which is the sum of the calculated masses, is approximately that estimated for ExoVIII from its SDS-PAGE mobility. To test this suggestion, we performed the following genetic experiments.

Two deletions were produced by *Nde*I digestion of pSJS74 (14). Both fused *recT* to the region between *ori* and *rop* of pBR322. One was predicted to reduce the mass of a RecE-RecT fusion protein by 18 kDa, and the other was predicted to reduce the mass by 23 kDa. The effects differ because the deletions differ by 4 base pairs at the *Nde*I junction. A third deletion fused *recT* to a region near the *int* gene of Rac and is expected to reduce the mass of a fusion protein by 23 kDa. A fourth deletion in pJC1509 removes 214 codons of *recT* and fuses the remaining 55 codons to 285 codons of *bla* (see legend to Fig. 1B). This should have added about 7.6 kDa of mass to ExoVIII. We inserted a *kan* cassette in both orientations into the *Pst*I cleavage site in *bla*. Neither the deletion nor insertion mutations in *recT* had any effect on the mobility of ExoVIII as visualized by Western blot of SDS-polyacrylamide gels (data not shown). Thus we have no genetic support for the fusion protein hypothesis.

DISCUSSION

Nucleotide sequencing has revealed two open reading frames in the region of the Rac prophage previously thought to be occupied by one (14, 29). Amino acid and genetic analysis had already shown that the longer is *recE*, which encodes ExoVIII nuclease (29). The shorter is herein named *recT* because deletion mutation analysis shows that it is required for conjugational recombination in a *recB recC* double mutant. Cloning *recE* and *recT* separately by using compatible plasmid vectors showed *recE* is also required for conjugational recombination in the same background.

Hall et al. (20) report that RecT protein has already been purified and been found to have single-stranded DNA renaturase activity like the beta protein of lambda. In this paper we have shown that *recT* can replace the *redB* gene which encodes the beta protein. As a result, we infer that the two proteins have similar in vivo functions. Thus, Rac encodes analogs of both Exo and Beta proteins, which have been implicated in lambda phage recombination (33, 37).

The sequence of *recE* is too short to encode a protein as massive as purified ExoVIII protein has been estimated to be (23, 27). Chu et al. (10) hypothesized that an SDS-denaturation-resistant portion of the protein might explain the high molecular mass estimates based on mobility in SDS-PAGE. Discovery of *recT* as a partially overlapping reading frame raised the possibility that ExoVIII was a fusion RecET protein. Genetic tests showed that this was not the case, however. In addition, R. Kolodner and coworkers (25) have performed detailed peptide mapping experiments with ExoVIII and RecT proteins and have shown that the two proteins have not "a single peptide in common." Deletion analysis shows that the middle third of *recE* may encode the portion of the protein responsible for the molecular mass overestimates.

Discovery that most of *recE* is 44% identical in nucleotide sequence to a portion of the b2 region of lambda confirms the very limited similarity observed between isofunctional regions of Rac and lambda phages, i.e., between *redB* and *recT* and between *redX* and *recE*. This *recE*-b2 region similarity raises interesting speculations concerning the evolution and function of these regions of Rac and lambda phages. For example, perhaps the b2 region is required for recombination in a bacterial host for lambda other than *Escherichia coli*. In rationalizing this idea, we could speculate that the b2 region might have diverged from a duplication of the ancestral *recE* region and specialized. Alternatively we could speculate that *recE* might be derived from an ancestral b2 region and converged in function to the *red* region of lambda.

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