Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 4622–4626, August 1984
Biochemistry

Molecular analysis of the *recF* gene of *Escherichia coli*

(nucleotide sequence/genetic recombination/DNA repair/DNA synthesis/maxicells)

MICHAEL A. BLANAR, STEVEN J. SANDLER, MARIA-EUGENIA ARMENGOD*, LLOYD W. REAM†, AND ALVIN J. CLARK

Department of Molecular Biology, University of California, Berkeley, CA 94720

Communicated by Franklin W. Stahl, March 13, 1984

ABSTRACT **We analyzed the nucleotide sequence of a 1.325-kilobase region of wild-type *Escherichia coli* containing a functional *recF* gene and six Tn*3* mutations that inactivate *recF*. The analysis shows a potentially translatable reading frame of 1071 nucleotides, which is interrupted by all six insertions. A protein of 40.5 kilodaltons would result from translation of the open reading frame, and a radioactive band of protein of an apparent molecular weight of ≈40 kilodaltons was seen by the maxicell method using a *recF*$^+$ plasmid. Putative truncated peptides were seen when two *recF*::Tn*3* mutant plasmids were used. Differential expression of *dnaN* and *recF* from a common promoter was noted. *recF332*::Tn*3* was transferred to the chromosome where, in hemizygous condition, it produced UV sensitivity indistinguishable from that produced by two presumed *recF* point mutations.**

The *recF* gene of *Escherichia coli* lies within a cluster of genes involved in DNA metabolism, the gene order being *gyrB recF dnaN dnaA* (1). *gyrB* has been shown to code for one of the subunits of DNA gyrase (2), *dnaN* determines the β subunit of DNA polymerase III holoenzyme (3), and *dnaA* encodes a 52-kilodalton (kDa) protein (4, 5) involved with the initiation of replication of the *Escherichia coli* chromosome (6).

The phenotype of a *recF* mutant indicates that *recF* also is involved in DNA metabolism and possibly with replication. UV irradiation of *recF143* mutants produces a wide variety of effects (e.g., refs. 7–9) economically interpretable by the hypothesis that induction of the *lexA* regulon is partially inhibited (10). Such induction is thought to depend in part on formation of single-strand gaps because of incomplete replication of UV-damaged DNA and in part on association of *recA*, *lexA*, and *cI* gene products with these gaps (11). Therefore, it is plausible that the *recF* gene product is involved in some way with formation of these gaps or association of single-stranded DNA with proteins. *recF143* strains also behave abnormally when exposed to treatments by other DNA-damaging agents [e.g., psoralen and visible light (12), thymine starvation (unpublished results), nalidixic acid (7, 13), and coumermycin (14)], leading one to the hypothesis that *recF*$^+$ genotype is required for normal SOS inducibility (15).

Recently, evidence has appeared that *recF143* substantially inhibits a variety of DNA metabolic events in unirradiated cells: plasmid recombination (16, 17) and one type of mismatch correction (ref. 18; unpublished results). *recF143* also substantially reduces conjugational recombination (7, 14, 19) and λ phage–prophage recombination (20) in special mutant backgrounds. These effects are plausibly explained by hypothesizing that *recF* encodes an endonuclease (21) or a protein involved in DNA synthesis. Cloning of the *recF* region has been described (22), and in this paper we identify the *recF* gene product.

MATERIALS AND METHODS

Bacterial Strains. All bacterial strains used are derivatives of *E. coli* K-12. Gene symbols are those used by Bachmann (23). JC11033 was produced by transforming the Hfr strain JC158 (24) with the plasmid pML2.

Plasmids. pJC605 is a 7.9-kilobase (kb) plasmid derived by cloning a 3.6-kb fragment carrying *recF*, called cJC1, with pBR322 (22). pMAB4 is a 3.6-kb plasmid derived by cloning the 1.3-kb *Eco*RI–*Pvu* II *recF*$^+$ fragment of cJC1 into the 2.3-kb *Eco*RI–*Pvu* II *bla*$^+$ fragment of pBR322. pJC655 is a 7.7-kb de(*bla*)300 ampicillin-sensitive (Amp^S) deletion mutant derivative of pJC605 (22). Tn*3* mutant derivatives of pJC655 (12.7 kb) were isolated by conduction with the conjugative plasmid pJC753. pJC753 is a Tn*3* insertion mutant of R1 drd-19·K1, a plasmid that has lost Tn*3* and Tn*4* by deletion and was produced upon transposition of Tn*3* from pJC752, a pSC101::Tn*3* derivative (unpublished results). pML2 is a 13.5-kb plasmid containing a 6.7-kb *Eco*RI *aphA*$^+$ fragment cloned into ColE1 (25). Construction of pSJS9, pSJS40, pSJS76, and pSJS77 using the temperature-dependent runaway-copy vector pBEU28 (26) will be published elsewhere.

Media, Materials, and Mutagenesis. Media, materials, and some methods have been described or cited by Ream and Clark (22). To detect transposon mutagenesis, JC13225, a strain carrying the target plasmid pJC655 and the conjugative plasmid pJC753 was used as donor, and JC9239 (*recF143*) was used as recipient in a 1-hr conjugation at 37°C. Colonies of Amp^R Tet^R (Amp- and tetracycline-resistant) [Met$^+$ Trp$^+$ Ile$^+$ Val$^+$] transconjugants appeared at frequencies of 3×10^{-8} and 1×10^{-7} per donor in two experiments while the conjugative plasmid was transmitted at 3×10^{-5} and 1×10^{-3}, respectively. Six UV^S transconjugants were found among 159 tested, indicating that the plasmid-borne *recF* gene was mutant. Hydroxylamine (H_2NOH) mutagenesis of plasmid DNA was performed *in vitro* as described by Humphreys *et al.* (27). Plasmid DNA (5 μg) was incubated with 1 M H_2NOH (in 1 mM EDTA) at 72°C for 30 min in one experiment and at 60°C for 15 min in another.

Sequence Determination. Sequencing was done essentially by the procedure of Maxam and Gilbert (28). 5′ termini were labeled with [γ-^{32}P]ATP (Amersham; >5000 Ci/mmol; 1 Ci = 37 GBq) and polynucleotide kinase (P-L Biochemicals). 3′ ends were labeled with the large fragment of DNA polymer-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Amp, ampicillin; Tet, tetracycline; Str, streptomycin; Kan, kanamycin; superscripts R, S, +, and −, resistant/resistance, sensitive/sensitivity, independent/independence, and dependent/dependence; kb, kilobase(s).

*Present address: Instituto de Investigaciones Citologicas, Amadeo de Saboya 4, Valencia-10, Spain.
†Present address: Department of Biology, Indiana University, Bloomington, IN 47405.

ase (Klenow; Boehringer Mannheim) and α-^{32}P-labeled deoxyribonucleoside triphosphates (Amersham; >3000 Ci/mmol).

Maxicells. Labeling with L-[^{35}S]methionine (Amersham; ≈1200 Ci/mmol) was performed essentially as described by Sancar *et al.* (29). Labeling was done at 42°C for 1 hr.

RESULTS

Tn*3* and Hydroxylamine-Induced Mutations. In order to ascertain the limits of the *recF* gene, we generated and characterized six insertion mutations of Tn*3* in the cloned *recF* gene. All the insertions resulted in the inability of the plasmid-borne *recF* gene to confer UV^R to an otherwise UV^S *recF143* chromosomal mutant strain. Each of the resultant plasmids complemented the temperature-sensitive mutation *dnaN608* (data not shown), indicating that none of these Tn*3* insertions eliminated *dnaN* function. Restriction enzyme mapping with *Bam*HI and *Hin*cII (30) was used to locate roughly and orient the transposon in each mutant insertion (data not shown). The six insertions occurred within a region of about 950 base pairs. Exact positions as determined by nucleotide sequencing are indicated in Fig. 1. As delimited by these Tn*3* insertion mutations, the *recF* gene and any other DNA fragment necessary for expression must extend from at least position 41 to position 983 in Fig. 1.

In addition to the Tn*3* insertion mutations, we also generated a nonconditional, H_2NOH-induced *recF* mutation, *recF289*. We transformed a *sup*$^+$ *recF143* strain, JC11810, with H_2NOH-treated DNA of pJC605 and selected for Tet^R. The resultant transformants were screened for sensitivity to 3.5 μg of nitrofurantoin per ml at 30°C and 42°C. Of 4500 transformants screened, 7 highly sensitive transformants were identified, and 1 of these, nitrofurantoin-sensitive at both temperatures, was found to contain a plasmid whose *recF* mutation could not be suppressed by various nonsense suppressors. This plasmid, pJC610, carries *recF289* and complements *dnaN608* (data not shown), demonstrating that it is *dnaN*$^+$.

Translocation of Plasmid-Carried *recF* Mutation. The plasmid-borne *recF* mutations were all detected in heterozygous condition with the chromosomal mutation *recF143*. To produce cells hemizygous for these mutations, we did the following: (*i*) transformed a *recF*$^+$ *dnaA508* (42°C^S) strain with the appropriate plasmid; (*ii*) selected and purified two spontaneous 42°C^R mutants of the resultant transformants to obtain plasmids that had integrated by homologous recombination in the *recF* region; (*iii*) incubated an inoculum from each clone at 30°C in the absence of antibiotics for 24 hr, followed by subculturing at 30°C for an additional 24 hr before screening individual clones for 42°C^S and for UV^S, to detect plasmid excision and either a hemizygous or homozygous *recF* mutation; and (*iv*) used the resultant isolates as donors in P1 transduction of the nearby *tnaA300*::Tn*10* marker, selecting Tet^R 42°C^R transductants and screening for UV^S, to isolate those that inherited the recipient's *dnaA*$^+$ and the donor's *recF* mutant allele. In steps *iii* and *iv*, we were able to isolate respectively *dnaA508* and *dnaA*$^+$ strains that were hemizygous for *recF289* and *recF332*::Tn*3*. Translocation was verified for the latter mutation by the Southern transfer method (31) using *Eco*RI digests of chromosomal DNA and pJC605 DNA as a labeled probe (data not shown). Strains hemizygous for *recF289* and *recF332*::Tn*3* were found to be as *UV*^{S} as the hemizygous *recF143* strain, JC11803 (data not shown). When crossed with donor JC11033, each produced essentially the same frequency of Thr$^+$ Leu$^+$ [Str^R (streptomycin-resistant) Ser$^+$] and Kan^R (kanamycin-resistant) [Str^R Ser$^+$] transconjugants as JC11803, both with chromosomal and plasmid pML2 markers, respectively (data not shown).

As the *recF*::Tn*3* mutations had been detected by their failure to complement *recF143*, we tested their ability to complement *recF289*. Strains carrying plasmids with the Tn*3* insertions at the extremes of the *recF* region and with *recF289* on the chromosome were UV^S, whereas those with *recF*$^+$ on the chromosome were UV^R.

DNA Sequence Analysis of the *recF* Gene. The nucleotide sequence of a 1.325-kb *Msp* I–*Eco*RI fragment, which has been shown to contain a completely functional *recF* gene (ref. 22; unpublished results) is presented in Fig. 1. It includes two long open reading frames. The shorter consists of 210 nucleotides and begins at the *Msp* I sequence. It encodes the carboxyl-terminal 70 amino acids of *dnaN* protein (32) and ends at a TAA that overlaps, by one nucleotide, the first ATG of the longer open reading frame. Beginning at this ATG, the latter consists of 1071 nucleotides, which could encode a 357-amino-acid protein of 40,519 daltons. The sequence A-A-T-G-A-G is centered 9 nucleotides prior to the A of the first ATG. Five of the 6 nucleotides of this sequence (underlined) are complementary with the 3′ end of *E. coli* 16S rRNA. Since the average ribosome-binding site has a 4.8-nucleotide complementarity centered 9.8 nucleotides from the A of an initiating ATG (33, 34), we hypothesize that the A-A-T-G-A-G sequence is a suitable ribosome-binding sequence. Thus, the longer open reading frame is potentially translatable and may be *recF*.

Maxicell Analysis of the recF Protein. We transformed pSJS9, pSJS40, pSJS76, and pSJS77 into an appropriate UV^S *recF143* recipient mutant strain and screened the Kan^R transformants for *recF*$^+$ complementation. The strains harboring pSJS9 and pSJS40 yielded UV^R colonies at 30°C, whereas those containing pSJS76 and pSJS77 remained UV^S (data not shown).

Using [^{35}S]methionine-labeled extracts from maxicells carrying these four plasmids, we obtained the autoradiograms shown in Fig. 2*b*. Temperature induction of pSJS9 (*recF*$^+$) for 1 hr results in the expression of four proteins with molecular masses of 59, 47, 43, and 29 kDa. Identities of these proteins have been confirmed by deletion analysis, and this evidence will be presented elsewhere. The 59-kDa protein is the result of a fusion of 93 codons of λ phage *exo* (35) with 446 codons of *dnaA* (36). The 47-kDa protein has not been identified, but it seems to be vector-encoded. The 43- and 29-kDa proteins are the *dnaN* and λ phage *bet* gene products, respectively (35). λ phage N protein was not observed in these experiments. It is of interest to note that all genes downstream of the temperature-induced *pL* and *pR* promoters produce substantial amounts of proteins, but that the *recF* gene product is not observed. We were successful in identifying the *recF* gene product only upon construction of deletion derivatives of pSJS9.

From the DNA sequence analysis, we expected pSJS40 (*recF*$^+$) to encode proteins of 7.2 and 43 kDa in addition to the 40.5-kDa recF protein. The smaller protein would result from a fusion of the 55 amino-terminal codons of λ phage *N* (35) to the 10 carboxyl-terminal codons of *dnaN* and would not be seen under the conditions we used. The larger protein would result from translation initiating at an ATG in the λ phage *N* gene, 23 codons upstream of the *recF* initiating codon. As shown in Fig. 2*b*, we observed two proteins of approximately the expected weights upon incubation at 42°C to initiate transcription from *pL* and *pR*. With the Tn*3* mutant plasmid pSJ76, we saw neither the 42- nor 40-kDa bands. Instead a new band appeared at 20.5 kDa. This is the size expected of the product of fusion between *recF* and the codons of Tn*3* in the appropriate reading frame. A larger peptide (24 kDa) with the additional 23 amino acids is probably obscured by the heavily labeled *cI* and *aphA* gene products. With plasmid pSJS77, we saw proteins of apparent molecular masses 36 kDa and 40 kDa as expected from the *recF* and upstream initiation codons, respectively.

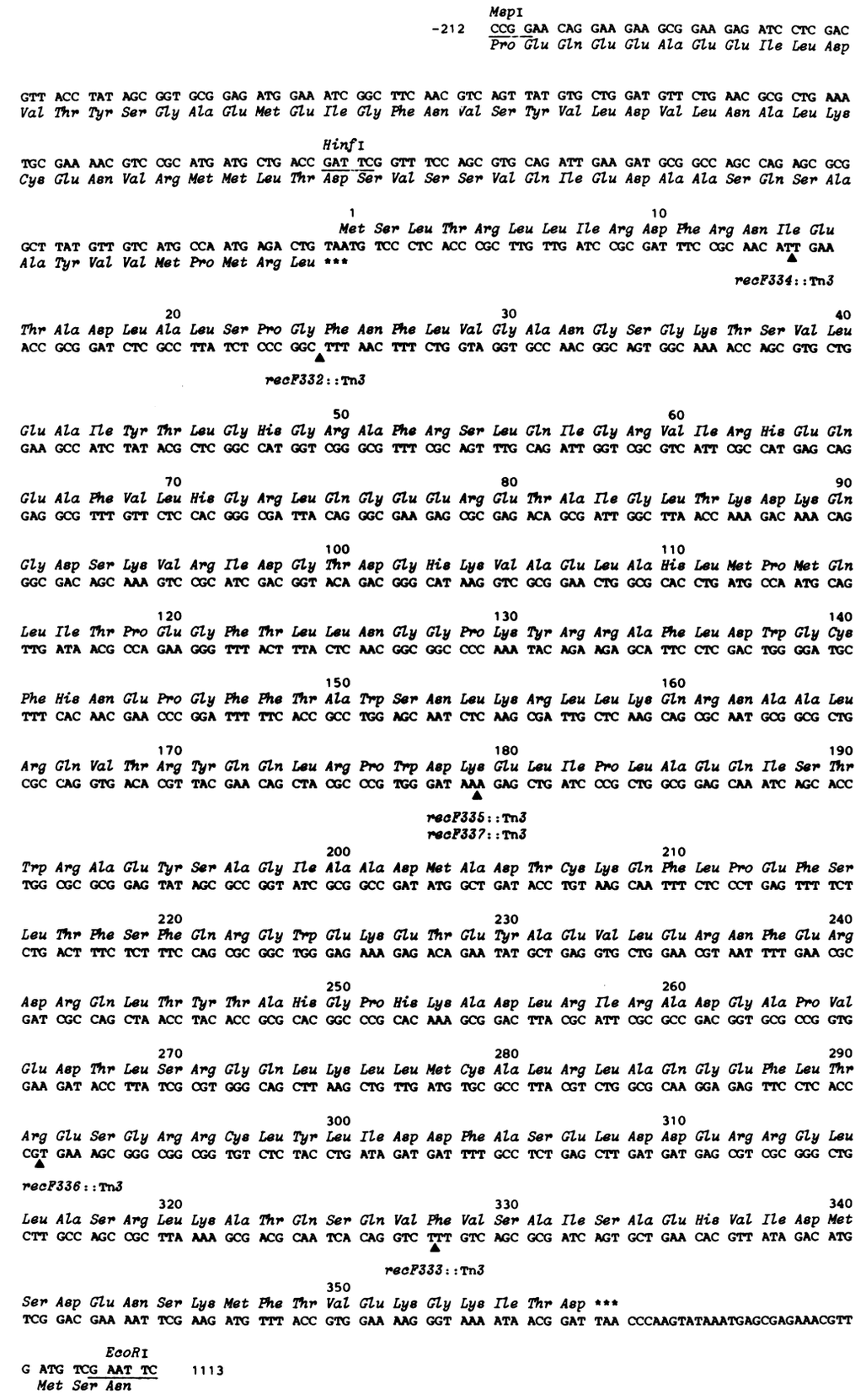

FIG. 1. DNA sequence of the *recF* gene and adjacent regions. The *recF* gene and the deduced amino acid sequence begin at position +1 and extend to the TAA termination codon at position 1074. The carboxyl-terminal sequence of *dnaN* extends from position −212 to +1, and the amino terminus of the *gyrB* gene begins at position 1103. Restriction endonuclease recognition sequences are underlined. ▲, Positions of insertion of the six Tn*3* mutations. Tn*3* elements were found in both orientations. In *recF334* and *recF336*, Tn*3* is oriented so that the *bla* gene is proximal to the *gyrB* side of *recF*, whereas *bla* is distal to the *gyrB* side in the insertion mutations *recF332*, *recF333*, *recF335*, and *recF337*. Description of the sequencing strategy is available upon request.

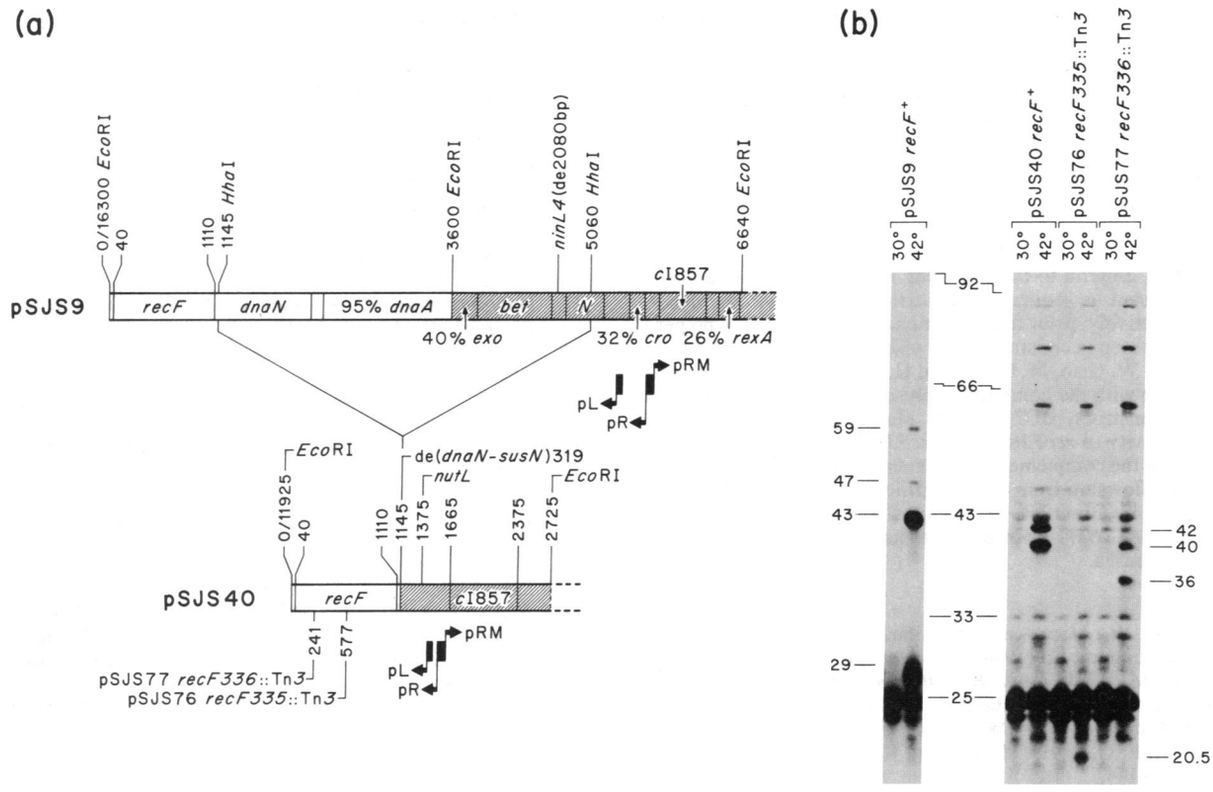

FIG. 2. (*a*) Features of the plasmids. The 3.6-kb *Eco*RI *recF*$^+$ fragment from pJC605 was cloned into a plasmid vector, constructed in this laboratory, in order to provide temperature-controlled transcription of cloned genes. pSJS9 contains the following λ phage genes: isodirectionally transcribing *pL* and *pR* promoters, the temperature-sensitive repressor *cI857*, a doubly amber *N* mutant (*N3*, *N57*), and *bet*$^+$. This plasmid was modified by partial digestion with *Hha* I to yield the plasmid pSJS40, from which 97% of *dnaN*, the remainder of *dnaA*, all of *bet* and part of *N* have been deleted. In addition, two insertion mutations, *recF335*::Tn*3* and *recF336*::Tn*3*, were substituted for *recF*$^+$ in pSJS40, yielding the *recF*$^-$ plasmids pSJS76 and pSJS77, respectively. Portions derived from λ phage are crosshatched. The pBEU28 vector (33) has been omitted. (*b*) Autoradiogram of [^{35}S]methionine-labeled extracts from maxicells. Protein extracts from cells containing the plasmids pSJS9, pSJS40, pSJS76, or pSJS77 were labeled at 30°C (noninduced) and 42°C (induced) and subjected to sodium dodecyl sulfate/12% polyacrylamide gel electrophoresis in two experiments; 100,000 cpm were loaded per lane. The gels were treated with fluor (EN^{3}HANCE, New England Nuclear) before they were dried and exposed to film for 40 hr at −70°C. Molecular weight marker positions of the following marker proteins are indicated by the center numbers: phosphorylase B, bovine serum albumin, ovalbumin, catalytic subunit of aspartate transcarbamoylase, and carbonic anhydrase. Numbers at the left and far right refer to those protein bands discussed within the text.

DISCUSSION

Almost all of the results described are consistent with the hypothesis that most of an open reading frame between *dnaN* and *gyrB* is *recF* and that the *recF* gene product is a 40.5-kDa protein. The exception is our observance of RecF$^+$ phenotype at 30°C (i.e., UV^R) without detecting *recF* gene product by maxicell analysis at this temperature. At present, we think there is too little *recF* gene product produced at 30°C to be detectable by the maxicell method. We were unable to determine the phenotype at 42°C because the plasmid vector we used is lethal at that temperature.

An analysis of the amino acid composition shows that the presumptive *recF* gene product contains 30.3% charged amino acids and has an isoelectric point of ≈7.6. A comparison of the primary structure of the *recF* gene product with various DNA binding proteins has not revealed any significant similarity.

Observations have indicated that *recF* and *dnaN* gene products are produced in substantially different amounts from a common promoter. In an earlier paper, Ream and Clark (22) were easily able to identify the *dnaN* product in minicells but were unable to identify a *recF* gene product. Other reports (4, 37) have identified only the *dnaA* and *dnaN* gene products from DNA of this region, although a complete *recF* gene was present in these studies.

There are three reasons that could explain this differential expression. First, it is possible that transcription terminates early in the *recF* sequence. A region of dyad symmetry begins at position 21 in Fig. 1 and ends at position 55 with a stem length of 13 nucleotide pairs and a loop distance of 9 nucleotides. This sequence may be a transcriptional pause sequence and a potential ρ-dependent terminator (38). Since UV irradiation suppresses polarity effects in the *gal* operon by reducing ρ activity (39, 40), the amount of *recF* gene product might increase after irradiation without having a *lexA* gene product binding site nearby (unpublished results). Expression of a putative pBEU28 gene downstream from *recF* and detectable by its 47-kDa protein product would not be affected by such a terminator perhaps because of the intervening *gyrB* promoter at the carboxyl-terminal end of *recF* (41). Second, it is possible that a *recF* transcript is cleaved by RNase action. A second region of dyad symmetry extends from position 305 through 338 (Fig. 1). The potential stem of an RNA structure from this region contains the sequence 5′-A-A-G-G-U-C, which is found in all symmetrical sequences of T7 early mRNAs cleaved by RNase III (42). Each of these two possibilities would result in truncated mRNAs, incapable of encoding a complete recF protein. Third, it is possible that translation of recF mRNA may be slow. The *recF* reading frame has a high percentage (43.3%) of nonoptimal codons (43), whereas the adjacent 70 codons of the carboxyl terminus of *dnaN* contain only 22% nonoptimal codons. All of these mechanisms may operate to prevent toxic effects due to overproduction of *recF* gene product.

An interesting feature of the presented sequence is the one-base overlap of *recF* and *dnaN*. Overlaps of one nucleotide are seen at the junctions of other genes in *E. coli* (44, 45) and have been hypothesized (45, 46) to couple translation of adjacent reading frames. This feature, with the neutralization of the limiting factors we have described, should allow for the overproduction and further characterization of the *recF* gene product.

We are very grateful to the following people: M. Gellert and H. Ohmori, who sent us sequence information on the *recF* and *dnaN* regions, respectively, prior to publication; Steven Polevoi, who performed the last step in construction of pSJS76 and pSJS77; M. Cherry, C. Gautier, W. Gish, M. Gouy, and H. Martinez for their invaluable assistance with the computer analyses; J. Nowak, for allowing us to use his construct, pJC753; and L. Margossian, who determined the UV sensitivity of *recF289* and *recF332*:Tn*3* hemizygous strains and performed the complementation experiments. M.-E.A. was supported by a fellowship from the Fundacion Juan March. This work was supported by Grant NP-237 from the American Cancer Society and by Grant AI 05371 from the National Institute of Allergy and Infectious Diseases.

1. Ream, L. W., Margossian, L., Clark, A. J., Hansen, F. G. & von Meyenburg, K. (1980) *Mol. Gen. Genet.* **180,** 115–121.
2. Gellert, M. (1981) *Annu. Rev. Biochem.* **50,** 879–910.
3. Burgers, P. M. J., Kornberg, A. & Sakakibara, Y. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5391–5395.
4. Hansen, F. G. & von Meyenburg, K. (1979) *Mol. Gen. Genet.* **175,** 135–144.
5. Fuller, R. S. & Kornberg, A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5817–5821.
6. Hirota, Y., Mordoh, J. & Jacob, F. (1970) *J. Mol. Biol.* **53,** 369–387.
7. Horii, Z. I. & Clark, A. J. (1973) *J. Mol. Biol.* **80,** 327–344.
8. Rothman, R. H. & Clark, A. J. (1977) *Mol. Gen. Genet.* **155,** 279–286.
9. Clark, A. J., Volkert, M. R., Margossian, L. J. & Nagaishi, H. (1982) *Mutat. Res.* **106,** 11–26.
10. McPartland, A., Green, L. & Echols, H. (1980) *Cell* **20,** 731–737.
11. Little, J. W. & Mount, D. W. (1982) *Cell* **29,** 11–22.
12. Sinden, R. R. & Cole, R. S. (1978) *J. Bacteriol.* **136,** 538–547.
13. Karu, A. E. & Belk, E. D. (1982) *Mol. Gen. Genet.* **185,** 175–182.
14. Smith, C. L. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2510–2513.
15. Witkin, E. M. (1976) *Bacteriol. Rev.* **40,** 869–907.
16. James, A. A., Morrison, P. T. & Kolodner, R. (1982) *J. Mol. Biol.* **60,** 411–430.
17. Cohen, A. & Laban, A. (1983) *Mol. Gen. Genet.* **189,** 471–474.
18. Fishel, R. A. & Kolodner, R. (1983) in *Cellular Responses to DNA Damage*, eds. Frieberg, E. C. & Bridges, B. A. (Liss, New York), pp. 309–324.
19. Gillen, J. R., Willis, D. K. & Clark, A. J. (1981) *J. Bacteriol.* **145,** 521–532.
20. Armengod, M.-E. (1981) *Mol. Gen. Genet.* **181,** 497–504.
21. Bresler, S. E., Krivonogov, S. V. & Lanzov, V. A. (1978) *Mol. Gen. Genet.* **166,** 337–346.
22. Ream, L. W. & Clark, A. J. (1983) *Plasmid* **10,** 101–110.
23. Bachmann, B. J. (1983) *Microbiol. Rev.* **47,** 180–230.
24. Bachmann, B. J. (1972) *Bacteriol. Rev.* **36,** 525–557.
25. Hershfield, V., Boyer, H. W. & Yanofsky, C. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3455–3459.
26. Uhlin, B. E., Schweickart, V. & Clark, A. J. (1983) *Gene* **22,** 255–265.
27. Humphreys, G. O., Willshaw, G. A. & Anderson, E. S. (1975) *Biochim. Biophys. Acta* **383,** 457–463.
28. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
29. Sancar, A., Hack, A. M. & Rupp, W. D. (1979) *J. Bacteriol.* **137,** 692–693.
30. Heffron, F., McCarthy, B. J., Ohtsubo, H. & Ohtsubo, E. (1979) *Cell* **18,** 1153–1163.
31. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
32. Ohmori, H., Kimura, M., Nagata, T. & Sakakibara, Y. (1984) *Gene* **28,** 159–170.
33. Shine, J. & Dalgarno, L. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1342–1346.
34. Steitz, J. A. (1979) in *Ribosomes: Structure, Function, and Genetics*, eds. Chambliss, G., Crowen, G. R., Davies, J., Davis, K., Kahan, L. & Nomura, M. (Univ. Park Press, Baltimore), pp. 479–495.
35. Daniels, D. L., Schroeder, J. L., Szybalski, W., Sanger, F., Coulson, A. R., Hong, G. F., Hill, D. F., Petersen, G. B. & Blattner, F. R. (1983) in *Lambda II*, eds. Hendrix, R. W., Roberts, J. W., Stahl, F. W. & Weisberg, R. A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 519–684.
36. Hansen, E. B., Hansen, F. G. & von Meyenburg, K. (1982) *Nucleic Acids Res.* **10,** 7373–7385.
37. Yuasa, S. & Sakakibara, Y. (1980) *Mol. Gen. Genet.* **180,** 267–273.
38. Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. L. (1978) *Nature (London)* **272,** 414–423.
39. Pollock, T. J., Tessman, E. S. & Tessman, I. (1979) *J. Bacteriol.* **138,** 122–125.
40. Fassler, J. S. & Tessman, I. (1980) *J. Bacteriol.* **144,** 1205–1207.
41. Menzel, R. & Gellert, M. (1984) *Cold Spring Harbor Symp. Quant. Biol.* **49,** in press.
42. Gegenheimer, P. & Apirion, D. (1981) *Microbiol. Rev.* **45,** 502–541.
43. Ikemura, T. (1981) *J. Mol. Biol.* **151,** 389–409.
44. Yanofsky, C., Platt, T., Crawford, I. P., Nichols, B. P., Christie, G. E., Horowitz, H., van Cleemput, M. & Wu, A. M. (1981) *Nucleic Acids Res.* **9,** 6647–6668.
45. Schumperli, D., McKenney, K., Sobieski, D. A. & Rosenberg, M. (1982) *Cell* **30,** 865–871.
46. Oppenheim, D. S. & Yanofsky, C. (1980) *Genetics* **95,** 785–795.