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Analysis of the *ruv* Locus of *Escherichia coli* K-12 and Identification of the Gene Product

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The *ruv* gene of *Escherichia coli*, which is associated with inducible mechanisms of DNA repair and recombination, has been cloned into the low-copy plasmid vector pHSG415. The recombinant plasmid pPVA101 fully complements the DNA repair-deficient phenotype of *ruv* mutants. Restriction endonuclease analysis of this plasmid revealed a 10.6-kilobase (kb) *Hin*dIII DNA insert which contained a 7.7-kb *Pst*I fragment identified as being from the chromosomal *ruv* region. Deletion analysis and Tn*1000* insertional inactivation of *ruv* function located the *ruv* coding region to a 2.2-kb section of the cloned DNA fragment. A comparison of the proteins encoded by *ruv* wild-type and mutant plasmids identified the gene product as a protein of molecular weight 41,000.

The *ruv* gene of *Escherichia coli* forms an essential component of the SOS system, a complex network of genes whose collective function helps cells survive damage to their DNA by promoting repair and moderating cell division while repair is effected (29, 34). As with other SOS genes, the expression of *ruv* is triggered by a variety of DNA lesions that have in common the ability to activate cleavage of LexA protein, the SOS repressor (13). Mutation of *ruv* increases sensitivity to mitomycin C, UV light, and ionizing radiation and leads to an unusual degree of filamentous growth of the damaged cells (11, 24). Filamentation can be suppressed by mutation of *sulA* or *sulB*, but this suppression does not increase resistance to UV light or mitomycin C (15, 25), which suggests that the inability to overcome SOS-induced inhibition of cell division is not the primary reason for radiation sensitivity, as it is in the case of *lon* mutants (4).

Recent studies have shown that *ruv* is essential for recombination in *recBC sbcB* mutants but not in wild-type strains, an observation which supports the view that *ruv* is involved in repair rather than in the control of division (15). Recombination in *recBC sbcB* strains depends on several other genes (*recA*, *recF*, *recJ*, *recN*, and *recQ*), most of which, like *ruv*, are also needed to promote recovery after damage to DNA (8, 18–20, 22, 26). At least two of these genes, namely, *recA* and *recN*, are regulated by LexA protein and are involved in the repair of DNA strand breaks (26, 34). There is no evidence to suggest that *ruv* function is needed to initiate recombination during the repair of UV lesions (24). This leaves the possibility that *ruv* defines an inducible activity which is needed to allow the exchanges initiated at lesions in DNA to proceed to a viable conclusion.

We previously reported the identification of recombinant λ phages and plasmids that complement the radiation sensitivity of *ruv* mutants (30). In this paper we describe a study of the cloned *ruv* gene by deletion analysis and insertional inactivation, and we report the identification of the gene product.

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MATERIALS AND METHODS

Strains and plasmids. The *E. coli* K-12 strains and plasmids used are listed in Table 1. Strain CS143 carries a mutation, *tls-1*, that confers temperature-sensitive growth in low-salt media. The mutant allele is 40 to 50% cotransducible with *eda* in the order *eda-ruv-tls-1* (unpublished data). pHSG415 is a low-copy-number vector which exhibits temperature-sensitive replication (7, 32). Strains carrying this plasmid or its derivatives were grown at 30°C. Recombinant phages λRL101, λRL102, λRL103, λRL104, and λRL105 are derived from a library constructed by ligating a partial *Sau*3A digest of *E. coli* DNA into *Bam*HI-digested DNA from λPE11, a *c*I857 derivative of the λL47 cloning vector (1). The ability of these phages to restore radiation resistance to *ruv* mutants has been described previously (30).

Growth media have been referred to previously (16, 17). LB broth and agar were supplemented as required with ampicillin or chloramphenicol at 25 μg/ml or kanamycin at 40 μg/ml. Procedures for strain construction by P1 transduction and conjugation have been described before, as have methods for determination of sensitivity to mitomycin C and measurement of survival after UV irradiation (14, 16, 17). Streptomycin was used in media for the selection of transconjugants at 100 μg/ml. Methods for working with phage λ were those of Silhavy et al. (31).

Enzymes and DNA analysis. Restriction endonucleases, T4 DNA ligase, and the Klenow fragment of DNA polymerase I were purchased from Bethesda Research Laboratories, Cambridge Biotechnology Laboratories, or Amersham International and were used as directed by the suppliers. Plasmids were maintained in strain AB2463 (*recA13*), and plasmid DNA was prepared by the rapid alkaline-sodium dodecyl sulfate lysis procedure of Ish-Horowicz and Burke (10). Chromosomal DNA was prepared by the method of Silhavy et al. (31). DNA restriction fragments were resolved by electrophoresis on 0.6 to 0.8% (wt/vol) agarose slab gels. Protocols for Southern hybridizations with ^{32}P-labeled probes and for other procedures referred to for DNA manipulation and analysis were those of Maniatis et al. (21) and Cohen et al. (3).

Identification of plasmid-encoded proteins. Recombinant plasmids were transformed into strain AB2480 (*uvrA recA*),

TABLE 1. *E. coli* strains and plasmids

Strain	Genotype (or relevant phenotype)	Source or reference
AB1157	F⁻ *thi-1 his-4 proA2 argE3 thr-1 leuB6 ara-14 lacY1 galK2 xyl-5 mtl-1 supE44 tsx-33 rpsL31*	2
AB2463	As AB1157 but *recA13*	2
AB2480	As AB1157 but *uvrA6 recA13*	9
HI24	As AB1157 but *ruvA4*	24
HI36	As AB1157 but *ruvB9*	24
N1373	F⁻ *ruv-51 thi-1 mtl-1 xyl-5 his-4 argE3 thrB1007 rpsL31 gyrA262 supE44 tsx-33*	30
N2057	As AB1157 but *ruv-60*::Tn*10*	30
N2058	As AB1157 but *ruv-59*::Tn*10*	30
CS81	As AB1157 but *eda-51*::Tn*10 ruv-52*	30
CS85	As AB1157 but *eda-51*::Tn*10 ruv-53*	30
CS86	As AB1157 but *eda-51*::Tn*10 ruv-54*	30
CS114	As N1373 but Δ*ruv-57 eda-51* Tc^s	30
CS115	As N1373 but Δ*ruv-58 eda-51* Tc^s	30
CS143	As AB1157 but *tls-1 eda-51* Tc^s	C. E. Shurvinton
W3110	F⁻ IN(*rrnD-rrnE*)*1*[a]	2
NH4104	F42 *lac*⁺/*uvrA6 proA2 leu-8 thr-4 his thi ara-14 lac-1*	K. B. Low
pHSG415	Ap^r Cm^r Km^r	H. M. Arthur (7)
pPVA101	*ruv*⁺ Km^s recombinant of pHSG415	30
pPVA105	*ruv*⁺; Cm^s deletion mutant of pPVA101	This work
pFB500	*ruv*⁺; deletion derivative of pPVA105	This work
pFB501	*ruv*⁺; deletion derivative of pFB500	This work
pFB502	*ruv* deletion derivative of pPVA101	This work

[a] IN, Inversion.

and proteins were labeled with [^{35}S]methionine by the maxicell technique of Sancar et al. (28) to specifically identify plasmid products. Proteins were separated on sodium dodecyl sulfate-polyacrylamide gels by the methods of West and Emmerson (35) and were visualized by fluorography.

RESULTS

The recombinant plasmid pPVA101 is able to correct the radiation sensitivity and recombination deficiency of *ruv* mutant strains (30). It carries a single *Hin*dIII insert common to the *ruv* transducing phages λRL101 and λRL103 (30). Strains lysogenized with these phages carry the prophage inserted at or very near *ruv* (unpublished data), which suggests that the phages contain DNA homologous to this region. In the case of λRL103, this possibility is supported by the fact that infection of a λ*c*⁺ lysogen of strain CS143 (*tls-1*) gives rise to colonies that grow at 42°C. Presumably, λRL103 carries *ruv* and at least part of the adjacent *tls*⁺ gene. We assume therefore that pPVA101 carries the chromosomal *ruv* region. Since pPVA101 cannot replicate at high temperature, we have been unable to test whether it also carries *tls*.

pPVA101 restores UV resistance to a range of *ruv* mutations. pPVA101 was transformed into a series of 10 strains representing a range of point mutations, deletions, and Tn*10* insertions in *ruv*. Ap^r Cm^r colonies were purified and examined for their sensitivity to UV light. In every case, the plasmid restored resistance to the level of *ruv*⁺ strains (Table 2). We assume therefore that pPVA101 probably carries the entire *ruv* gene.

Location by deletion of the *ruv* coding region. A series of single, double, and triple restriction enzyme digests established that pPVA101 consists of a 10.6-kilobase (kb) *Hin*dIII DNA insert within the 7.1-kb pHSG415 vector (Fig. 1a). This estimate of the size of the insert is lower than was previously reported (30) and is based on a more accurate sum of the sizes of the subfragments. Deletion derivatives of pPVA101 were tested to see if they retained the ability to correct the *ruv* defect. The deletions generated are shown in Fig. 1b. pPVA105 was formed by digestion of pPVA101 with *Eco*RI followed by religation. This procedure deleted 0.9 kb of the insert with the contiguous 1.7-kb fragment extending into the Cm^r region of the vector. pFB500 was constructed by digesting pPVA105 with *Bgl*II and *Eco*RI and filling in the resulting 3'-OH recessed ends by the Klenow polymerase reaction before religation. This method eliminated the 1.8-kb *Bgl*II-*Eco*RI fragment of pPVA105 and removed both enzyme recognition sites. A further deletion, pFB501, was made by digesting pFB500 with *Bam*HI to remove 0.5 kb of the vector and the adjacent 1.3 kb of insert DNA. Finally, plasmid pFB502 was generated by deleting the 4.0-kb *Kpn*I fragment from within the insert of pPVA101. Of the four deletion mutants generated, only pFB502 had lost the ability to restore resistance to UV light and mitomycin C. Examples of the survival of UV-irradiated *ruv* strains carrying deletion derivatives of pPVA101 are shown in Table 2 and Fig. 2a and b. We concluded that *ruv* must be in the 6.6-kb *Bam*HI-*Bgl*II DNA fragment of pPVA101 (Fig. 1a) and that the *Kpn*I site in this segment must be very close to or within the coding region.

Insertional inactivation of *ruv*. The limits of the *ruv* coding

TABLE 2. Survival of UV-irradiated strains carrying *ruv* wild-type or mutant plasmids

Strain	Rate of survival[a] in strain with Plasmid					
	pHSG415	pPVA101	pPVA105	pFB500	pFB501	pFB502
AB1157 (*ruv*⁺)	0.36	0.10				
HI24 (*ruvA4*)	0.00018	0.36	0.23	0.10	0.50	0.00011
HI36 (*ruvB9*)	0.00015	0.48	0.44	0.16	0.50	0.00013
N1373 (*ruv-51*)	0.000025	0.11		0.11		0.002
CS81 (*ruv-52*)	0.000047	0.44	0.31			
CS85 (*ruv-53*)	0.00033	0.34	0.16	0.10	0.18	0.00056
CS86 (*ruv-54*)	0.00025	0.37		0.07	0.15	0.00002
CS114 (*ruv-57*)	0.000041	0.11		0.51	0.50	
CS115 (*ruv-58*)	0.000041	0.43		1.0	0.2	0.001
N2058 (*ruv-59*)	0.000028	0.14	0.13	0.14	0.3	
N2057 (*ruv-60*)	0.00013	0.19	0.14	0.22	0.34	

[a] Values relate to the number of colonies formed after exposure to 60 J/m^{-2} of UV light expressed as a fraction of unirradiated controls.

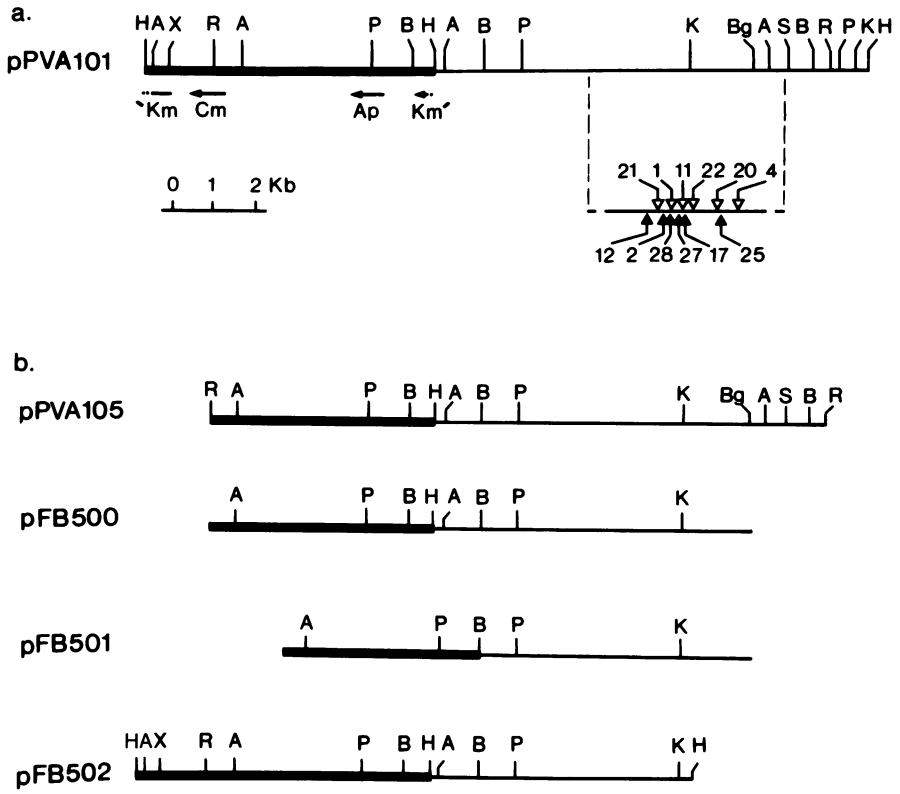

FIG. 1. Restriction maps of pPVA101 (a) and pPVA101 deletions (b). The pHSG415 vector is indicated by heavy lines, and the arrows in panel a show the positions of the ampicillin, chloramphenicol, and (interrupted) kanamycin resistance genes. The pHSG415 used in this work was found to carry an *Ava*I site in addition to that reported by Hashimoto-Gotoh et al. (7). All plasmids are aligned with respect to the *Kpn*I site within the *ruv* coding region of the *E. coli* DNA insert. The *ruv* coding region is defined in panel a by the numbered Tn*1000* insertions in the γδ (▽) or δγ (▲) orientation (6). Restriction endonuclease cleavage sites are abbreviated as follows: A, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; R, *Eco*RI; S, *Sal*I; X, *Xho*I.

region in pPVA101 were defined more accurately by locating Tn*1000* insertions that inactivated *ruv* function. Although the pHSG415 vector is nonconjugative and nonmobilizable (7, 32), it can be transferred during F-mediated conjugation as a cointegrate structure with F. The cointegrate is formed as an intermediate during transposition of the Tn*1000* element carried by the F plasmid (6). Resolution of the cointegrate in the recipient leaves the transferred plasmid with a randomly inserted copy of Tn*1000* (6).

pPVA101 was transformed into strain NH4104 (F42*lac*), and several single-colony isolates were used as donors in matings with F^- strains. Initial studies with strain HI24 (*ruvA4*) as recipient failed to identify any *ruv* mutant plasmids. More than 5,000 Ap^r Cm^r (*rpsL*) transconjugant colonies were examined, but all proved resistant to mitomycin C. Control experiments established that inactivation of the antibiotic resistance genes occurred with reasonable frequency. Thus, 25 of 300 Cm^r transconjugants tested were Ap^s, while 9 of 300 Ap^r clones were Cm^s. The possibility that cotransfer of F42*lac* masks the *ruv* mutant phenotype in transconjugants was ruled out by studies which revealed that *ruv* mutants carrying F42*lac* alone are just as sensitive to mitomycin C and UV light as are the isogenic F^- strains (data not shown). Therefore, we suspected that *ruv* function may somehow be required to allow recovery of viable plasmid products following cointegrate transfer. Further matings were conducted with the ruv^+ strain AB1157 as recipient. Ap^r Cm^r (*rpsL*) transconjugants were selected and pooled. Plasmid DNA was extracted and transformed into the *ruv* mutant HI24. Of 300 Ap^r Cm^r transformants tested, 39 were sensitive to both UV light and mitomycin C. Restriction analysis of the plasmid DNA prepared from these 39 clones revealed in each case a 5.7-kb Tn*1000* DNA insert (6) within the 10.6-kb *Hin*dIII fragment of pPVA101. The precise locations and orientations of the Tn*1000* inserts were determined with *Eco*RI and *Xho*I single digests. Twelve separate insertion sites were distinguished (Fig. 1a), and these sites defined a 2.2-kb region of pPVA101 spanning the *Kpn*I site identified by deletion analysis as being close to or within *ruv*.

Tn*1000* isolates 2, 4, 11, and 12 of pPVA101, which give a spread along the *ruv* coding region (Fig. 1a), were transformed into our collection of *ruv* mutants. In each case the strain constructed remained sensitive to UV light. However, we observed somewhat variable survival with independent cultures of the same strain grown from single colonies. Examples of the results obtained are shown in Fig. 2c and d. The increased survival observed in certain cases was due to subpopulations of resistant cells in the cultures used. Analysis of the surviving colonies revealed that they were fully resistant to mitomycin C, even when cured of the plasmid by growth at 42°C. Presumably, these are ruv^+ cells that arise through recombination between the plasmid and chromosomal alleles and that outgrow the *ruv* mutant cells. Previous studies established that *ruv* mutants have reduced viability and grow much more slowly than ruv^+ cells (15; unpublished data). The incidence of recombination has not been examined in detail, but the proportion of mitomycin C-resistant

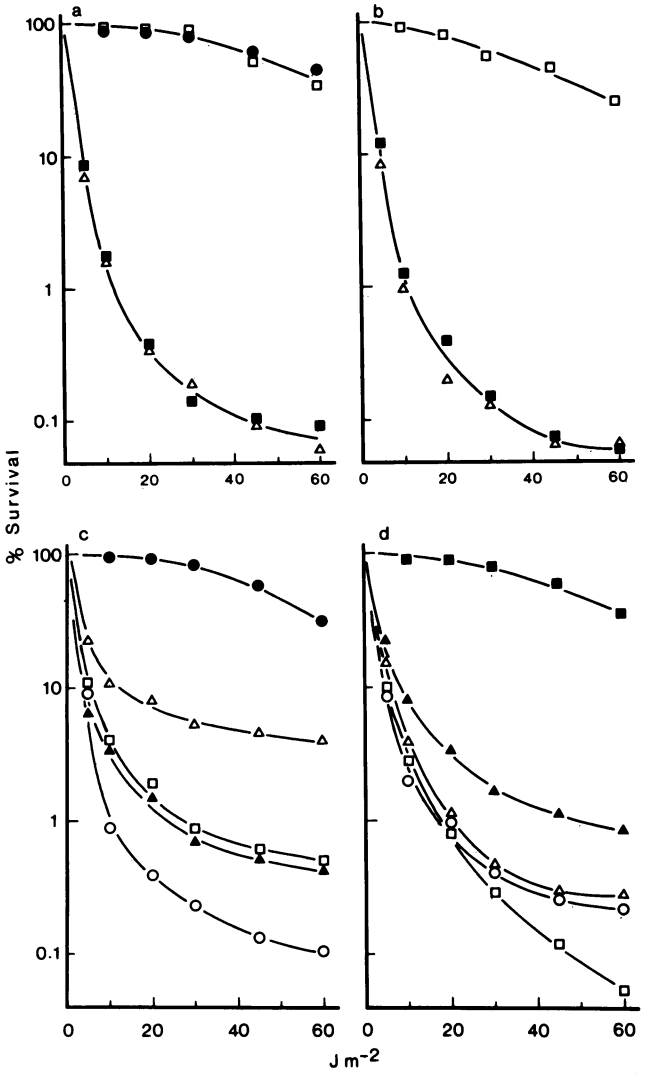

FIG. 2. Survival of UV-irradiated strains carrying *ruv* wild-type and mutant plasmids. The plasmid strains used were (a) AB1157 (*ruv*$^+$)pHSG415, ●; HI24 (*ruvA4*)pHSG415, ■; HI24 pFB501, □; HI24 pFB502, △; (b) CS85 (*ruv-53*)pHSG415, ■; CS85 pFB501, □; CS85 pFB502, △; (c) HI24 pPVA101, ●, or pPVA101*ruv*::Tn*10* insertions 2, 4, 11, or 12, shown as ▲, ○, □, or △, respectively; (d) CS85 pPVA101, ■, or pPVA101*ruv*::Tn*10* insertions 2, 4, 11, or 12, shown as ▲, ○, □, or △, respectively. Selection of the plasmid was maintained at all times by use of the appropriate antibiotic.

cells in independent cultures has been shown to vary from less than 1% to more than 50% of the total (data not shown). The inactivation of the pPVA101 *ruv* gene by Tn*1000* insertion also prevents the plasmid from restoring normal levels of recombination and F-prime repliconation in a *recBC sbcB ruv* mutant (data not shown). From these studies, we conclude that a single coding region probably defines both the DNA repair and the recombination activities associated with *ruv*.

Overproduction of *ruv* product may be lethal. Although *ruv* is flanked in pPVA101 by a number of convenient restriction sites (Fig. 1a), we have been unable so far to subclone the intact gene into the multicopy vectors pBR322 or pACYC184 (21), either with selection for *ruv* function or without. The 5.2-kb *Bam*HI-*Hin*dIII fragment of pFB502 (Fig. 1b), which contains the section of *ruv* to the left of the *Kpn*I site (Fig. 1a), has been cloned into pACYC184. Similarly, the 2.4-kb *Kpn*I-*Sal*I fragment of pPVA101, which carries the remainder of *ruv*, has been cloned into pUC19, a derivative of pBR322 (23, 33). These plasmid constructs have no effect on the sensitivity of *ruv* mutants to mitomycin C or UV light (data not shown). Since they cover the whole of the coding region as defined by insertions, it appears that the *ruv* DNA does not contain any sequence that is deleterious when present in multiple copies. We suspect therefore that the failure to subclone the intact gene is due to a lethal effect associated with increased synthesis of the gene product.

pPVA101 carries the chromosomal *ruv* region. The restriction map in Fig. 1a suggests that the chromosomal *ruv* gene should be located on a 7.7-kb *Pst*I DNA fragment. To test this possibility, we examined the DNA of a strain with a Tn*10* insertion in the chromosomal *ruv* gene (30). In this strain the *Pst*I fragment containing *ruv* should be increased in size by the 9.3 kb of Tn*10* DNA (12), since Tn*10* is devoid of *Pst*I recognition sites. Chromosomal DNA extracted from *ruv*$^+$ and *ruv*::Tn*10* strains was digested to completion with *Pst*I. The products were separated by electrophoresis, transferred to nitrocellulose, and probed with the 1.5-kb *Kpn*I-*Bgl*II fragment of *ruv* from the insert in pPVA101. The probe hybridized to a 7.7-kb band in the *ruv*$^+$ DNA and an approximately 17-kb band in the *ruv*::Tn*10* DNA (data not shown).

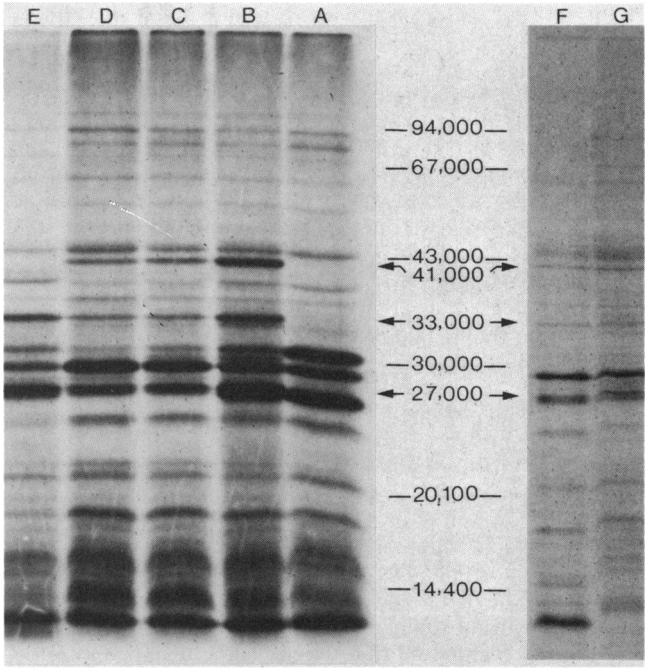

FIG. 3. Proteins encoded by pPVA101 and its deletion derivatives. Proteins were labeled with [^{35}S]methionine in maxicell preparations of the plasmid strains, separated on 15% polyacrylamide-sodium dodecyl sulfate gels, and visualized by fluorography. A, pHSG415 vector; B, pPVA101; C, pPVA105; D, pFB500; E, pFB502; F, pFB500; G, pFB501. Molecular weight standards were phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and α-lactalbumin (14,400). The arrows locate the 41,000, 33,000, and 27,000 proteins referred to in the text. The 27,000 protein in the pPVA101 and pFB502 lanes is somewhat obscured by the slightly faster-migrating product of the chloramphenicol resistance gene.

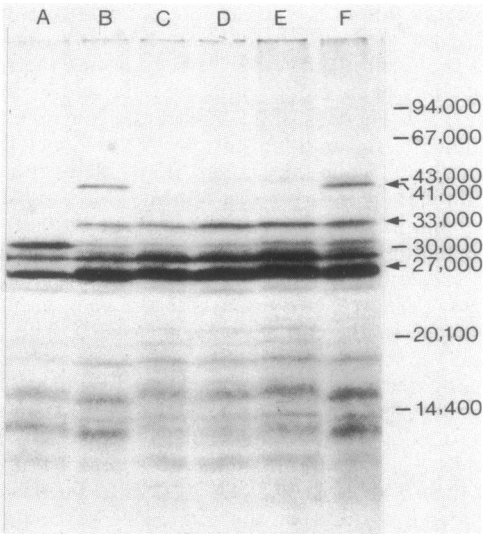

FIG. 4. Proteins encoded by pPVA101*ruv*::Tn*1000* plasmids. A, pHSG415; B, pPVA101; C, pPVA101::Tn*1000*-4; D, pPVA101::Tn*1000*-11; E, pPVA101::Tn*1000*-12; F, pPVA101. Molecular weight standards are as in Fig. 3.

Identification of the *ruv* product. The *ruv*$^+$ plasmids pPVA101, pPVA105, pFB500, and pFB501 all encode three major proteins with molecular weights of approximately 27,000, 33,000, and 41,000 which are not found in extracts from a strain carrying pHSG415 (Fig. 3). The 27,000 and 33,000 proteins are also produced in cells harbouring pFB502, which is deleted for part of the *ruv* coding region (Fig. 1b), but the 41,000 protein is absent. This fact suggested that the 41,000 protein might be a product of the *ruv*$^+$ region of the DNA insert in pPVA101. The results (Fig. 4) reveal that the 41,000 protein is also absent from strains carrying the pPVA101*ruv*::Tn*1000* mutants, whereas the 27,000 and 33,000 proteins are still produced. We examined each one of the *ruv*::Tn*1000* insertions depicted in Fig. 1a and found that all gave the same protein profile, which confirms that the 41,000 protein is encoded by *ruv*.

DISCUSSION

We have investigated the structure of a recombinant plasmid capable of restoring resistance to UV light and mitomycin C to *ruv* mutants. Deletion studies of pPVA101 and the mapping of Tn*1000* insertions identified a 2.2-kb section of DNA that is essential for complementing *ruv*. This section is located within a 7.7-kb *Pst*I fragment that hybridizes to chromosomal DNA defined by Tn*10* insertions as being from the *ruv* region. We conclude that pPVA101 carries the chromosomal *ruv*$^+$ gene. Sections of DNA that flank the *ruv* region and that extend some distance into the vector can be deleted from pPVA101 without affecting the ability to correct the *ruv* mutant phenotype. Furthermore, Tn*1000* insertions flanking the *ruv* coding region in pPVA101 do not appear to interfere with expression of *ruv* (data not shown). The cloned gene is therefore probably transcribed from its own promoter.

A comparison of the proteins encoded by wild-type and mutant plasmids revealed that the *ruv* region specifies a polypeptide with a relative molecular weight of 41,000. Assuming an average amino acid composition, a protein of this size could be encoded by about 0.9 kb of DNA, which is less than half our estimate of 2.2 kb for the *ruv* coding region. It is possible that the *ruv* region encodes a second protein which is made in very small amounts or which cannot be distinguished from other plasmid-encoded products separated by electrophoresis. However, all of the Tn*1000* insertions in pPVA101 that inactivate *ruv* function prevent synthesis of the same 41,000 protein. Therefore, while the possibility of two adjacent genes cannot be ruled out at this stage, especially since insertions can exert polar effects (12), we favor the idea that *ruv* defines a single locus.

A rather intriguing observation made during the course of this study is that when we employed an *ruv* mutant strain as a recipient in matings with an F-prime donor carrying pPVA101, we were unable to recover *ruv*::Tn*1000* plasmids among the transconjugants, although insertions in regions flanking *ruv* were quite common. There was no difficulty in recovering insertions within *ruv* when the recipient used was *ruv*$^+$. These observations suggest that a functional *ruv* product may be necessary to allow recovery of viable products from cointegrate molecules, at least after their conjugational transfer. Although Tn*1000* encodes its own activity for resolving transpositional cointegrates (5, 27), this fact does not exclude the possibility that other activities are needed for viable resolution in vivo. *ruv* has already been shown to be essential in certain genetic backgrounds for the recovery of viable recombinants from standard Hfr crosses (15).

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LITERATURE CITED

1. **Arthur, H. M., D. Bramhill, P. Eastlake, and P. T. Emmerson.** 1982. Cloning of the *uvrD* gene of *E. coli* and identification of the product. Gene **19**:285–295.
2. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
3. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R factor DNA. Proc. Natl. Acad. Sci. USA **69**:2110–2114.
4. **George, J., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *E. coli*. III. Mutations in *sfiA* and *sfiB* restore division in *tif* and *lon* strains and permit the expression of mutator properties of *tif*. Mol. Gen. Genet. **140**:309–332.
5. **Grindley, N. D. F.** 1983. Transposition of Tn*3* and related transposons. Cell **32**:3–5.
6. **Guyer, M. S.** 1978. The γδ sequence of F is an insertion sequence. J. Mol. Biol. **126**:347–365.
7. **Hashimoto-Gotoh, T., F. C. H. Franklin, A. Nordheim, and K. N. Timmis.** 1981. Specific-purpose cloning vectors. I. Low copy number, temperature-sensitive, mobilisation-defective pSC101-derived containment vectors. Gene **16**:227–235.
8. **Horii, Z. I., and A. J. Clark.** 1973. Genetic analysis of the *recF* pathway of genetic recombination in *Escherichia coli* K12: isolation and characterisation of mutants. J. Mol. Biol. **80**:327–344.
9. **Howard-Flanders, P., R. P. Boyce, and L. Theriot.** 1966. Three loci in *Escherichia coli* K12 that control the excision of pyrimidine dimers and certain other mutagen products. Genetics **53**:1119–1136.
10. **Ish-Horowicz, D., and J. F. Burke.** 1981. Rapid and efficient cosmid cloning. Nucleic Acids Res. **9**:2989–2998.
11. **Iyehara, H., and N. Otsuji.** 1975. Ultraviolet light sensitivity of *Escherichia coli* K-12 strains carrying *ruv* mutations in combination with *uvrA* or *lon* mutant alleles. J. Bacteriol. **121**:735–736.

12. **Kleckner, N.** 1981. Transposable elements in prokaryotes. Annu. Rev. Genet. **15**:341–404.
13. **Little, J. W., D. W. Mount, and C. R. Yanisch-Perron.** 1981. Purified *lexA* protein is a repressor of the *recA* and *lexA* genes. Proc. Natl. Acad. Sci. USA **78**:4199–4203.
14. **Lloyd, R. G., and S. D. Barbour.** 1974. The genetic location of the *sbcA* gene of *Escherichia coli*. Mol. Gen. Genet. **134**: 157–171.
15. **Lloyd, R. G., F. E. Benson, and C. E. Shurvinton.** 1984. Effect of *ruv* mutations on recombination and DNA repair in *Escherichia coli* K12. Mol. Gen. Genet. **194**:303–309.
16. **Lloyd, R. G., and B. Low.** 1976. Some genetic consequences of changes in the level of *recA* gene function in *Escherichia coli* K12. Genetics **84**:675–695.
17. **Lloyd, R. G., B. Low, G. N. Godson, and E. A. Birge.** 1974. Isolation and characterization of an *Escherichia coli* K-12 mutant with a temperature-sensitive RecA$^-$ phenotype. J. Bacteriol. **120**:407–415.
18. **Lloyd, R. G., S. M. Picksley, and C. Prescott.** 1983. Inducible expression of a gene specific to the recF pathway for recombination in *Escherichia coli* K12. Mol. Gen. Genet. **167**:162–167.
19. **Lovett, S. T., and A. J. Clark.** 1983. Genetic analysis of regulation of the recF pathway of recombination in *Escherichia coli* K-12. J. Bacteriol. **153**:1471–1478.
20. **Lovett, S. T., and A. J. Clark.** 1984. Genetic analysis of the *recJ* gene of *Escherichia coli* K-12. J. Bacteriol. **157**:190–196.
21. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Nakayama, H., K. Nakayama, N. Irono, Y. Nakayama, and P. C. Hanawalt.** 1984. Isolation and genetic characterization of a thymineless death-resistant mutant of *Escherichia coli* K12: identification of a new mutation (*recQ1*) that blocks the RecF recombination pathway. Mol. Gen. Genet. **195**:474–480.
23. **Norrander, J., T. Kempe, and J. Messing.** 1983. Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene **26**:101–106.
24. **Otsuji, N., H. Iyehara, and Y. Hideshima.** 1974. Isolation and characterization of an *Escherichia coli ruv* mutant which forms nonseptate filaments after low doses of ultraviolet light irradiation. J. Bacteriol. **117**:337–344.
25. **Otsuji, N., and H. Iyehara-Ogawa.** 1979. Thermoresistant revertants of an *Escherichia coli* strain carrying *tif-1* and *ruv* mutations: non-suppressibility of *ruv* by *sfi*. J. Bacteriol. **138**:1–6.
26. **Picksley, S. M., P. V. Attfield, and R. G. Lloyd.** 1984. Repair of DNA double-strand breaks in *Escherichia coli* K12 requires a functional *recN* product. Mol. Gen. Genet. **195**:267–274.
27. **Reed, R. R.** 1981. Transposon-mediated site-specific recombination: a defined *in vitro* system. Cell **25**:713–719.
28. **Sancar, A., R. P. Wharton, S. Seltzer, B. M. Kacinski, N. D. Clarke, and W. D. Rupp.** 1981. Identification of the *uvrA* gene product. J. Mol. Biol. **148**:45–62.
29. **Shurvinton, C. E., and R. G. Lloyd.** 1982. Damage to DNA induces expression of the *ruv* gene of *Escherichia coli*. Mol. Gen. Genet. **185**:352–355.
30. **Shurvinton, C. E., R. G. Lloyd, F. E. Benson, and P. V. Attfield.** 1984. Genetic analysis and molecular cloning of the *Escherichia coli ruv* gene. Mol. Gen. Genet. **194**:322–329.
31. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Timmis, K. N.** 1981. Gene manipulation in vitro, p. 49–109. *In* S. W. Glover and D. A. Hopwood (ed.), Genetics as a tool in microbiology. Cambridge University Press, Cambridge.
33. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
34. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
35. **West, S. C., and P. T. Emmerson.** 1977. Induction of protein synthesis in *Escherichia coli* following UV or γ-irradiation, mitomycin C treatment or *tif* expression. Mol. Gen. Genet. **151**:57–67.