Resolution of Holliday Junctions in *Escherichia coli*: Identification of the *ruvC* Gene Product as a 19-Kilodalton Protein

GARY J. SHARPLES AND ROBERT G. LLOYD*

*Department of Genetics, University of Nottingham, Queens Medical Centre,
Nottingham NG7 2UH, United Kingdom*

The *ruvC* gene of *Escherichia coli* specifies a nuclease that resolves Holliday junction intermediates in genetic recombination (B. Connolly, C. A. Parsons, F. E. Benson, H. J. Dunderdale, G. J. Sharples, R. G. Lloyd, and S. C. West, Proc. Natl. Acad. Sci. USA 88:6063–6067, 1991). The gene was located between *aspS* and the *ruvAB* operon by DNA sequencing and deletion analysis of *ruvC* plasmids and was shown to encode a protein of 18,747 Da. Analysis of the DNA flanking *ruvC* indicated that the gene is transcribed independently of the LexA-regulated *ruvAB* operon and is not under direct SOS control. *ruvC* lies downstream of an open reading frame, *orf-33*, for a protein which migrates during sodium dodecyl sulfate-polyacrylamide gel electrophoresis as a 33-kDa polypeptide. These two genes probably form an operon. However, expression of *ruvC* was found to be very poor relative to that of *orf-33*. A double ribosomal frameshift between these genes is proposed as a possible reason for the low level of RuvC. Two further open reading frames of unknown function were identified, one on either side of the *orf-33–ruvC* operon.

Much of our understanding of the enzymatic mechanisms of genetic recombination has come from studies of the RecA protein of *Escherichia coli*. In vitro, RecA catalyzes homologous pairing and strand exchange between DNA duplexes in reactions that are primed by regions of single-stranded DNA (22). An essential intermediate in these reactions is a heteroduplex joint, or Holliday junction, in which the two DNA duplexes are held together by the exchanged strands. Recombinant molecules are produced by specific endonucleolytic cleavage of this intermediate at the point of exchange (18, 19). Connolly and West (6) detected a nuclease with this ability in *E. coli* cell extracts by assaying for specific cleavage of Holliday junctions in an ongoing strand exchange reaction driven by RecA. The nuclease activity was found to be dependent on *ruvC* (5).

The *ruv* locus is known to be involved with DNA repair and recombination. Mutations at this locus increase sensitivity to UV light, mitomycin, and ionizing radiation (16, 20) and reduce the efficiency of recombination, particularly in *recB recC sbcB sbcC* and *recB recC sbcA* genetic backgrounds (16, 17). In the wild type, the involvement of *ruv* in recombination is masked to a large degree by the product of *recG*. Thus, recombination proceeds reasonably efficiently in *ruv* or *recG* single mutants but is blocked when both genes are inactivated (15). The double mutants are also as sensitive to UV light as *recA* mutants.

Three genes have been identified at the *ruv* locus (25). *ruvA* and *ruvB* form a single operon (4, 28) which is regulated by LexA as part of the SOS response (30, 32). These two genes encode proteins of 22 and 37 kDa, respectively, both of which have been purified. RuvA is a DNA-binding protein, while RuvB is an ATPase (13, 29). Complementation studies identified a third gene, *ruvC* (25). Mutations at this locus confer a phenotype which is very similar to that of *ruvA* or *ruvB* mutants.

Nucleotide sequencing of the *ruvC* region. Our previous studies (25) located *ruvC* in the region immediately upstream

of the *ruvAB* operon. We determined the nucleotide sequence of this upstream region, using the plasmid constructs shown in Fig. 1. pFB511 and pFB512 carry inserts extending from *aspS* to just beyond *ruvA* cloned in pBR322 and pUC18, respectively (25). The two Tn*1000* insertions in pFB511 (γδ29 and γδ31) were described previously by Sharples et al. (25). pGS746 was made by excising the 2.3-kb *Hinc*II-*Eco*RV(1) fragment from pFB512 and inserting it into the *Sma*I site of pTZ19R (Pharmacia LKB). pGS751 and pGS760 carry the same insert cloned in the pGEM-7Zf(+) and pGEM-7Zf(−) vectors, respectively, from Promega. pGS762 was made by cutting the 1.12-kb *Stu*I-*Eco*RV(1) fragment from pGS760 with *Stu*I and *Hind*III and inserting it into pUC18 (33) cut with *Sma*I and *Hind*III. pGS765 was made by excising the insert in pGS760 Δ8A (hereafter referred to as Δ8A) with *Hinc*II and *Hind*III and directing it into pUC18 cut with *Sma*I and *Hind*III. A functional *ruvC* gene in the plasmids constructed was detected by complementation analysis of the UV and mitomycin sensitivity of *E.*
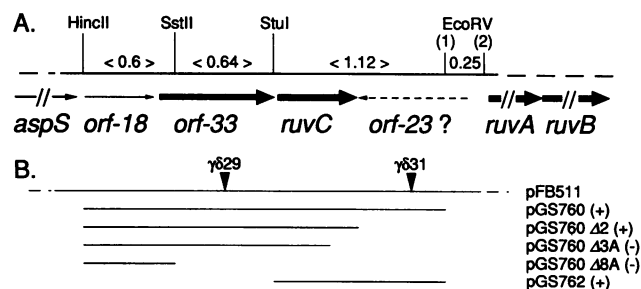

FIG. 1. (A) Restriction map showing the molecular organization of the genes in the *ruv* region of the *E. coli* chromosome. The distances between restriction sites are shown in kilobases. The flanking *aspS* and *ruvAB* genes are interrupted for reasons of scale. (B) DNA inserts carried by the recombinant plasmids used. The inserts are aligned with respect to the restriction sites as shown above. Plasmids are identified on the right, along with their ability (+) or inability (−) to make *ruvC* strains resistant to UV light. The deletion derivatives of pGS760 were made by using exonuclease III.

* Corresponding author.

```
HincII *****     (*****)
GTCAACGAAGAGGCAGCGTGAAGGATAAAGTGTATAAGCGTCCCGTTTCGATCTTAGTGGTCATCTACGCACAAGATACGAAACGGGTGCTGATGTTGCA  100
     orf-18  M K D K (V) Y K R P V S I L V V I Y A Q D T K R V L M L Q

GCGGCGTGACGATCCCGATTTCTGGCAGTCGGTAACCGGCAGCGTGGAAGAGGGTGAAACCGCGCCGCAAGCTGCCATGCGCGAAGTAAAGGAAGAGGTC  200
 R R R D D P D F W Q S V T G S V E E G E T A P Q A A M R E V K E E V

ACCATTGATGTTGTCGCTGAACAACTGACCTTAATTGACTGTCAGCGCACGGTAGAGTTTGAAATTTTTTCACATTTACGTCATCGCTATGCGCCGGGCG  300
 T I D V V A E Q L T L I D C Q R T V E F E I F S H L R H R Y A P G V

TGACGCGTAATACGGAATCATGGTTCTGTCTTGCGCTTCCGCACGAGCGGCAGATCGTTTTCACTGAACATCTGGCTTACAAGTGGCTTGATGCGCCTGC  400
 T R N T E S W F C L A L P H E R Q I V F T E H L A Y K W L D A P A
                                 -35              -10        >>>>>>> <<<<<<<      *****
TGCGGCGGCGCTCACTAAGTCCTGGAGCAACCGGCAGGCGATTGAACAGTTTGTAATTAACGCTGCCTGAACAGGCAGGGCTATCTGGAGATATTTTTAT  500
 A A A L T K S W S N R Q A I E Q F V I N A A .                        orf-33 M
                                                                        <Δ8A
GGCAGGTCATAGTAAATGGGCCAACACCAGACATCGTAAAGCTGCGCAGGATGCTAAGCGCGGTAAAATCTTCACTAAAATCATTCGTGAGCTGGTAACC  600
 A G H S K W A N T R H R K A A Q D A K R G K I F T K I I R E L V T
SstII
GCGGCTAAGCTGGGCGGTGGCGATCCGGACGCTAACCCGCGTCTGCGTGCGGCGGTGGATAAAGCACTGTCTAACAACATGACCCGTGACACACTGAACC  700
 A A K L G G G D P D A N P R L R A A V D K A L S N N M T R D T L N R

GCGCAATTGCACGTGGTGTGGGCGGTGATGATGGCGCAAACATGGAAACCATCATCTACGAAGGTTACGGTCCTGGCGGCACGGCAATCATGATTGAATG  800
 A I A R G V G G D D D A N M E T I I Y E G Y G P G G T A I M I E C

TCTGTCTGACAACCGCAACCGTACCGTTGCTGAAGTGCGTCATGCATTTAGCAAATGTGGCGGTAACCTCGGTACTGATGGTTCCGTTGCCTATCTGTTC  900
 L S D N R N R T V A E V R H A F S K C G G N L G T D G S V A Y L F
       ><  δγ29
AGCAAAAAAGGCGTGATCTCCTTCGAGAAAGGCGATGAAGACACCATCATGGAAGCAGCACTGGAAGCAGGTGCTGAAGACGTTGTGACCTATGATGACG 1000
 S K K G V I S F E K G D E D T I M E A A L E A G A E D V V T Y D D G

GCGCGATTGATGTCTACACTGCATGGGAAGAAATGGGTAAAGTGCGCGACGCTCTGGAAGCGGCAGGTCTGAAAGCAGACAGCGCGGAAGTTTCCATGAT 1100
 A I D V Y T A W E E M G K V R D A L E A A G L K A D S A E V S M I

CCCGTCTACCAAAGCTGATATGGATGCAGAAACCGCACCGAAACTGATGCGTCTGATCGATATGCTGGAAGATTGCGACGACGGTGCAGGAAGTTTACCAT 1200
 P S T K A D M D A E T A P K L M R L I D M L E D C D D V Q E V Y H
                                              StuI        *****
AACGGTGAAATCTCTGATGAGGTCGCAGCGACTCTCTGATGAGGCCTGCTAAACAGCAAAACGGAGACGCGTGATGGCTATTATTCTCGGCATTGATCCG 1300
 N G E I S D E V A A T L .                       ruvC M A I I L G I D P
                 orfII M R P A K Q Q N G D A ***
GGTTCGCGCGTGACCGGCTACGGCGTCATCCGCCAGGTAGGTAGGCAACTGTCCTACCTGGGTAGCGGATGCATCCGCACCAAAGTGGATGATTTACCGT 1400
 G S R V T G Y G V I R Q V G R Q L S Y L G S G C I R T K V D D L P S

CTCGTCTGAAACTCATCTATGCGGGCGTGACGGAAATCATCACCCAGTTCCAGCCTGATTATTTCGCCATTGAACAAGTCTTTATGGCAAAGAACGCTGA 1500
 R L K L I Y A G V T E I I T Q F Q P D Y F A I E Q V F M A K N A D

CTCAGCCCTGAAACTGGGCCAGGCGCGCGGCGTGGCGATTGTGGCGGCGGTGAATCAGGAGTTGCCAGTATTTGAATACGCGGCACGTCAGGTAAAGCAA 1600
 S A L K L G Q A R G V A I V A A V N Q E L P V F E Y A A R Q V K Q
           <Δ3A
ACGGTGGTAGGTATTGGCAGTGCCGAAAAAAGCCAGGTGCAGCATATGGTCCGCACCTTGCTGAAACTGCCCGCTAATCCACAGGCGGATGCCGCCGATG 1700
 T V V G I G S A E K S Q V Q H M V R T L L K L P A N P Q A D A A D A
                                                                        <Δ2
CGCTGGCGATTGCTATCACCCACTGCCACGTTAGTCAGAATGCGATGCAGATGAGCGAATCGCGGCTGAACCTGGCGAGAGGGCGACTGCGTTAAGTTAT 1800
 L A I A I T H C H V S Q N A M Q M S E S R L N L A R G R L R .   AATA
                                                                        .
ACCGCCTCGGTCAGTTCCGGCTGAGGCGTTTCCACTCCCTCCGCATGAATCAACACCAAACCCGGATGATGCCACAAACTGGCTGGCGTGACGGTTTTAC 1900
TGGCGGAGCCAGTCAAGGCCGACTCCGCAAAGGTGAGGGAGGCGTACTTAGTTGTGGTTTGGGCCTACTACGGTGTTGACCGACGCACTGCCAAAATG
 V A E T L E P Q P T E V G E A H I L V L G P H H W L S A P T V T K

GCTCCCACGGAATAGAACCCAGGAACCAGAATTTCCAGAAAGTGAGTTTTGCATTCGGATTGCTATTTAACAATTCTCCAAACGTCTCTATTTCACCCAC 2000
CGAGGGTGCCTTATCTTGGGTCCTTGGTCTTAAAGGTCTTTCACTCAAAACGTAAGCCTAACGATAAATTGTTAAGAGGTTTGCAGAGATAAAGTGGGTG
 R E W P I S G L F W F K W F T L K A N P N S N L L E G F T E I E G V

CGGAATACATAGCGCTTCTTTATAAATATCAAAAACAAATTTTGAACAGAACTGGCGCGAAGATTCGTATTTAAAACCGGTGTGGTAAAGTTTGCGTAGC 2100
GCCTTATGTATCGCGAAGAAATATTTATAGTTTTTGTTTAAAAACTTGTCTTGACCGCGCTTCTAAGCATAAATTTTGGCCACACCATTTCAAACGCATCG
 P I C L A E K Y I D F V F K S C F Q R S S E Y K F G T H Y L K R L
           ><  γδ31
CGGGAGGGAACCTGTTCAACAATTCGTTGTTTTTGTCGTTCTGTCAGCCCAGCGTCTAATCGCTTTATAGCATAGCGTTGATTAGACGAACGTTTAATAA 2200
GCCCTCCCTTGGACAAGTTGTTAAGCAACAAAAACAGCAAGACAGTCGGGTCGCAGATTAGCGAAATATCGTATCGCAACTAATCTGCTTGCAAATTATT
 R S P V Q E V I R Q K Q R E T L G A D L R K I A Y R Q N S S R K I

AACGAGATAGCGTGGTGATGGTTGAAAGGGGAACCCGGCTTTCTGCAACCAGGAAGTCTTCACCGTTATGACCGATAATGATCCCGACGTGATTACTCCA 2300
TTGCTCTATCGCACCACTACCAACTTTCCCCTTGGGCCGAAAGACGTTGGTCCTTCAGAAGTGGCAATACTGGCTATTACTAGGGCTGCACTAATGAGGT
 F R S L T T I T S L P V R S E A V L F D E G N H G I I I G V H N S W
                                                        EcoRV(1)
GCAATTTGATGCAGCTGATATTTGACCAAATAAGGCAGCACCTATACATGTAAAGACGATATCTCCAATTTCATATTCAGCAGGGTAATTAATATTCACG 2400
CGTTAAACTACGTCGACTATAAACTGGTTTATTCCGTCGTGGATATGTACATTTCTGCTATAGAGGTTAAAGTATAAGTCGTCCCATTAATTATAAGTGC
 C N S A A S I Q G F L A A G I C T F V I D G I E Y E A P Y N I N M

TTGTCATTCCATTGAAATAGATACAAATATACGTAATAGCATAAAAAATTTATTTTTTCAGGAGGCGCAATGTATATTTTTATTACGCATTTCTTCACTG 2500
AACAGTAAGGTAACTTTATCTATGTTTATATGCATTATCGTATTTTTTAAATAAAAAAGTCCTCCGCGTTACATATAAAAATAATGCGTAAAGAAGTGAC
 **** < orf-23
                                          ••••••••••••••• SOS box 1
AATATGTAATATTAAAATATTTGCTTCCAATATAACCTGTAGAATAAATTATACTGTGCCATTTTTCAGTTCATCGAGACACCTCGCAAGTTTTCTTCAT 2600
TTATACATTATAATTTTATAAACGAAGGTTATATTGGACATCTTATTTAATATGACACGGTAAAAAGTCAAGTA
   -10            -35     -10              -35

  •••EcoRV(2)•••••• SOS box 2                    *****
CCTTCGCTGGATATCTATCCAGCATTTTTTATCATACAGCATTATCTTTGATTCATTACGCAGGAGCGTCATGTGATAGGCAGACTCAGAGGCATCATC 2700
   -35            -10                              ruvA M I G R L R G I I
```

FIG. 2. Nucleotide sequence of the *ruvC* region. The 2,363-bp sequence from the *HincII* site to the *Eco*RV(1) site is as determined in this work. The sequence from *Eco*RV(1) to base 2700 is taken from the work of Benson et al. (4) and is included to facilitate reading of the entire *orf-23* sequence and to show the position of *ruvC* relative to the *ruvAB* operon. Translation of the open reading frames described in this work is shown below the sequence. The valine in parentheses defines an alternative translational start codon in *orf-18*. Putative promoter regions are underlined and labelled −10 and −35 above the sequence. Potential ribosome-binding sites are marked with asterisks. Chevrons indicate areas of inverted repeats referred to in the text. ><, Tn*1000* insertions γδ29 and γδ31; <, last base deleted from the *Eco*RV(1) end in Δ2 and Δ3A. The two dotted 16-bp sequences upstream of *ruvA* are the LexA binding sites described by Benson et al. (4).

coli K-12 strain CS85 (*ruvC53*) or N1373 (*ruvC51*) (25). These two strains are derivatives of AB1157 [F⁻ Δ(*gpt-proA*)62 *leuB6 ara-14 lacYl rpsL31 thi-1 his-4 argE3 thr-1 kdgK51 rfbD*(?) *galK2 xyl-5 mtl-1 tsx-33 supE44*] (1, 2, 31). Nucleotide sequencing was done by the dideoxynucleotide chain-termination method (24) with a T7 sequencing kit from Pharmacia LKB and single-stranded DNA extracted from strain JM101 [F'128 *proAB⁺ lacIᵠ ZΔM15 traD36*/Δ(*lac-pro*) *endA1 hsdR17 gyrA96 supE44*] (33) carrying the appropriate plasmid. pGS746, pGS751, pGS760, and a series of exonuclease III deletion derivatives were used to provide a series of overlapping sequences of both strands. Exonuclease III deletions were generated with the Erase-A-Base system from Promega. Where suitable deletions were not available, oligonucleotide primers based on sequences already determined were used to provide the required overlaps. γδ29 and γδ31 insertions in pFB511 were located by sequencing the appropriate restriction fragments subcloned in pGEM-7Zf(+) and pGEM-7Zf(−).

The nucleotide sequence determined for the 2,363-bp *Hinc*II-*Eco*RV(1) fragment is shown in Fig. 2. The 3' end of the sequence is contiguous with the sequence containing *ruvAB* reported by Benson et al. (4). For the purpose of analysis, Fig. 2 also shows the sequence from *Eco*RV(1) to the beginning of the *ruvA* gene. The new sequence revealed an overlap of 628 bp at the 5' end with the sequence reported by Eriani et al. (11). The region of overlap lies downstream of the aspartyl-tRNA synthetase gene (*aspS*) and contains the end of the intergenic repeat unit identified by Sharples and Lloyd (26). Our sequence differs from that presented by Eriani et al. (11) at four positions (bp 411, 438, 449, and 617) but matches exactly the sequence of this region obtained independently by Shinagawa (27).

Identification of *ruvC*. pGS760 carries the intact *ruvC⁺* gene as defined by its ability to make *ruvC* mutants fully resistant to UV light (data not shown). Deletion analysis of this construct located the DNA encoding *ruvC* between the *Stu*I site and bp 1798, the endpoint of Δ2 (Fig. 1B). The sequence in this region reveals an open reading frame (*orf*) of 521 bp extending from a putative ATG initiation codon at bp 1274 to a TAA stop codon at bp 1793. The 5'-GGAGA-3' sequence located just upstream provides a possible ribosome-binding site. Translation of this open reading frame would produce a polypeptide with a predicted molecular mass of 18,747 Da. A protein of about this size is produced by pGS760 and all the 3' deletion derivatives up to and including Δ2 (Fig. 3 and data not shown). However, synthesis of this protein is eliminated by Δ3A, which removes 175 bp from the 3' end of the reading frame. We conclude that the 19-kDa protein is the product of *ruvC*. This protein has been expressed from the *lac* promoter in pGS762, purified, and shown to be the product of the reading frame identified by sequencing of the amino terminus (9).

***ruvC* forms an operon with *orf-33*.** The DNA encoding *ruvC* lies downstream of an open reading frame extending from an ATG initiation codon at bp 599 to a TGA stop codon at bp 1237. We believe this to be the gene identified previously as *orf-33* (25). It produces a protein of 26,425 Da, which migrates during sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as a 33-kDa polypeptide (Fig. 3, Δ2 and Δ3A). The expression of this protein is prevented by insertion of a kanamycin resistance cassette at the *Sst*II site and by a deletion in pGS760 extending from *Eco*RV(1) to bp 1036 (results not shown). Sharples et al. (25) also showed that expression of this protein is prevented by
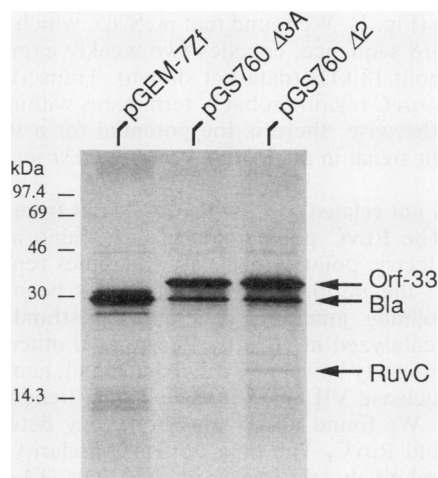

FIG. 3. Fluorograph showing plasmid-encoded proteins labelled in maxicells (23) with [³⁵S]methionine and separated by SDS-PAGE. Strain AB2480 (as strain AB1157 but *uvrA6 recA13*) (12) transformed with the plasmid indicated was grown to an A_{650} of 0.5 in 5 ml of M9 medium supplemented with ampicillin (100 μg/ml), irradiated with 45 J of UV light per m², and then incubated in the dark for 1 h at 37°C. Cycloserine was added to a final concentration of 200 μg/ml, and incubation continued for a further 16 h. The cells were pelleted, washed, and resuspended in 1 ml of M9 medium lacking MgSO₄ and amino acids. A 10-μCi amount of L-[³⁵S]methionine was added and incubation was continued at 37°C before the cells were washed to remove unincorporated label, resuspended in 50 μl of SDS gel loading buffer (2% SDS, 20% [vol/vol] glycerol, 5% β-mercaptoethanol, 0.2% bromophenol blue), and heated for 3 min at 100°C. Samples (10 to 20 μl) were then electrophoresed through a 15% polyacrylamide gel containing 0.2% SDS, and the labelled proteins were visualized by fluorography. The positions of molecular mass markers are indicated in kilodaltons on the left. The plasmids used are identified above each lane.

insertion γδ29, which we located between bp 918 and 919 (Fig. 2).

A possible promoter for *orf-33* is located at bp 431 to 458, though the proposed −35 region is not good. The sequence 5'-GGAGA-3' provides a possible ribosome-binding site. A 16-bp sequence extending across the proposed −35 region from bp 422 to 437 has some similarity with LexA binding sites (CTGN₁₀CAG) found in the promoters of SOS-inducible genes (32). However, we have been unable to detect any increase in the expression of *orf-33* (or of the downstream *ruvC* gene) following induction of the SOS response by mitomycin (data not shown). The *orf-33* promoter may also serve to express *ruvC*. There is no obvious sequence that would terminate transcription at the end of *orf-33*. A short peptide of 11 amino acids encoded between *orf-33* and *ruvC* may provide a means for translational coupling of the two genes. Such an intricate link involving a double ribosomal frameshift would be consistent with the very low level of expression of *ruvC* relative to that of *orf-33* (Fig. 3). However, *ruvC⁺* plasmids that carry deletions of the *orf-33* promoter region retain the ability to complement *ruvC* mutations (data not shown). These multicopy constructs probably express *ruvC* from plasmid promoters, but we cannot rule out the possibility of weak secondary promoters in the DNA insert remaining 5' to *ruvC*. The *orf-33–ruvC* operon is flanked by two other open reading frames, *orf-18* on the 5' side and the convergent *orf-23* on

the 3' side (Fig. 1). We found that pGS765, which carries the entire *orf-18* sequence, encodes two weakly expressed proteins of about 18 kDa (data not shown). Transcription from the *orf-33–ruvC* region probably terminates within the *orf-23* region. Otherwise, there is the potential for a very strong termination signal in nucleotides 2604 to 2631 just upstream of *ruvA*.

RuvC is not related to Holliday resolvases from phages T4 and T7. The RuvC polypeptide of 173 amino acids has a basic isoelectric point of pH 9.59. In studies reported elsewhere (9), the purified RuvC protein has been shown to cleave Holliday junctions generated in strand exchange reactions catalyzed in vitro by RecA. Two other nucleases with this property have been studied in detail, namely, phage T4 endonuclease VII (14, 18) and phage T7 endonuclease I (7, 8, 19). We found no obvious homology between these proteins and RuvC. The only obvious similarity is that all three are relatively small basic polypeptides; T4 endonuclease VII has a molecular mass of 18 kDa (3, 14), while T7 endonuclease I has a mass of 17 kDa (10, 21). The sequence Phe-Glu-Tyr occurs in all three proteins and may be of significance, but the zinc finger motif identified in T4 endonuclease VII (3) is not present in either RuvC or T7 endonuclease I. A search of the NBRF-PIR data base (release 27) showed no substantial homologies between RuvC and any other proteins.

Nucleotide sequence accession number. The sequence of *ruvC* and the flanking region has been submitted to EMBL and has been given the accession number X59551.

REFERENCES

1. **Attfield, P. V., F. E. Benson, and R. G. Lloyd.** 1985. Analysis of the *ruv* locus of *Escherichia coli* K-12 and identification of the gene product. J. Bacteriol. **164**:276–281.
2. **Bachmann, B. J.** 1987. Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12, p. 1190–1219. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
3. **Barth, K. A., D. Powell, M. Trupin, and G. Mosig.** 1988. Regulation of two nested proteins from gene 49 (recombination endonuclease VII) and of a λ RexA-like protein of bacteriophage T4. Genetics **120**:329–343.
4. **Benson, F. E., G. T. Illing, G. J. Sharples, and R. G. Lloyd.** 1988. Nucleotide sequencing of the *ruv* region of *Escherichia coli* K-12 reveals a LexA regulated operon encoding two genes. Nucleic Acids Res. **16**:1541–1549.
5. **Connolly, B., C. Parsons, F. E. Benson, H. J. Dunderdale, G. J. Sharples, R. G. Lloyd, and S. C. West.** 1991. Resolution of Holliday junctions in vitro requires *Escherichia coli* ruvC gene product. Proc. Natl. Acad. Sci. USA **88**:6063–6067.
6. **Connolly, B., and S. C. West.** 1990. Genetic recombination in *E. coli*: Holliday junctions made by RecA protein are resolved by fractionated cell-free extracts. Proc. Natl. Acad. Sci. USA **87**:8476–8480.
7. **DeMassy, B., R. A. Weisberg, and F. W. Studier.** 1987. Gene 3 endonuclease of bacteriophage T7 resolves conformationally branched structures in double-stranded DNA. J. Mol. Biol. **193**:359–376.
8. **Dickie, P., G. McFadden, and A. R. Morgan.** 1987. The site-specific cleavage of synthetic Holliday junction analogs and

related branched DNA structures by bacteriophage T7 endonuclease I. J. Biol. Chem. **262**:14826–14836.
9. **Dunderdale, H. J., F. E. Benson, C. A. Parsons, G. J. Sharples, R. G. Lloyd, and S. C. West.** The formation and resolution of recombination intermediates by *E. coli* RecA and RuvC proteins. Nature (London), in press.
10. **Dunn, J. J., and F. W. Studier.** 1983. Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. J. Mol. Biol. **166**:477–535.
11. **Eriani, G., G. Dirheimer, and J. Gangloff.** 1990. Aspartyl-tRNA synthetase from *Escherichia coli*: cloning and characterization of the gene, homologies of its translated amino acid sequence with asparaginyl- and lysyl-tRNA synthetases. Nucleic Acids Res. **18**:7109–7118.
12. **Howard-Flanders, P., L. Theriot, and J. B. Stedeford.** 1969. Some properties of excision-defective recombination-deficient mutants of *Escherichia coli* K-12. J. Bacteriol. **97**:1134–1141.
13. **Iwasaki, H., T. Shiba, K. Makino, A. Nakata, and H. Shinagawa.** 1989. Overproduction, purification, and ATPase activity of the *Escherichia coli* RuvB protein involved in DNA repair. J. Bacteriol. **171**:5276–5280.
14. **Kosak, H. G., and B. W. Kemper.** 1990. Large-scale preparation of T4 endonuclease VII from over-expressing bacteria. Eur. J. Biochem. **194**:779–784.
15. **Lloyd, R. G.** 1991. Conjugational recombination in resolvase-deficient *ruvC* mutants of *Escherichia coli* K-12 depends on *recG*. J. Bacteriol. **173**:5414–5418.
16. **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.
17. **Lloyd, R. G., C. Buckman, and F. E. Benson.** 1987. Genetic analysis of conjugational recombination in *Escherichia coli* K-12 strains deficient in RecBCD enzyme. J. Gen. Microbiol. **133**:2531–2538.
18. **Müller, B., C. Jones, B. Kemper, and S. C. West.** 1990. Enzymatic formation and resolution of Holliday junctions in vitro. Cell **60**:329–336.
19. **Müller, B., C. Jones, and S. C. West.** 1990. T7 endonuclease I resolves Holliday junctions formed in vitro by RecA protein. Nucleic Acids Res. **18**:5633–5636.
20. **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.
21. **Parsons, C., and S. C. West.** 1990. Specificity of binding to four-way junctions in DNA by bacteriophage T7 endonuclease 1. Nucleic Acids Res. **18**:4377–4384.
22. **Radding, C. M.** 1991. Helical interactions in homologous pairing and strand exchange driven by RecA protein. J. Biol. Chem. **266**:5355–5358.
23. **Sancar, A., A. M. Hack, and W. D. Rupp.** 1979. Simple method for identification of plasmid-coded proteins. J. Bacteriol. **137**:692–693.
24. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
25. **Sharples, G. J., F. E. Benson, G. T. Illing, and R. G. Lloyd.** 1990. Molecular and functional analysis of the *ruv* region of *Escherichia coli* K-12 reveals three genes involved in DNA repair and recombination. Mol. Gen. Genet. **221**:219–226.
26. **Sharples, G. J., and R. G. Lloyd.** 1990. A novel repeated DNA sequence located in the intergenic regions of bacterial chromosomes. Nucleic Acids Res. **18**:6503–6508.
27. **Shinagawa, H.** Personal communication.
28. **Shinagawa, H., K. Makino, M. Amemura, S. Kimura, H. Iwasaki, and A. Nakata.** 1988. Structure and regulation of the *Escherichia coli* ruv operon involved in DNA repair and recombination. J. Bacteriol. **170**:4322–4329.
29. **Shinagawa, H., T. Shiba, H. Iwasaki, K. Makino, T. Takahagi, and A. Nakata.** 1991. Properties of the *Escherichia coli* RuvA and RuvB proteins involved in DNA repair, recombination and mutagenesis. Biochimie **73**:505–508.
30. **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.

31. **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.

32. **Walker, G. C.** 1984. Mutagenesis and inducible responses to

deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.

33. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.