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An *E.coli* RuvC mutant defective in cleavage of synthetic Holliday junctions

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ABSTRACT

***Escherichia coli* RuvC protein is a specific endonuclease that resolves recombination intermediates into viable products. The structural features needed for RuvC activity were investigated by sequencing three *ruvC* mutations and relating the base pair changes identified to the activity of the mutant proteins. Each of the three mutations is a single base-pair substitution. *ruvC51* converts glycine-15 to an aspartic acid residue. The product of *ruvC51* was purified and shown to retain the ability to bind junctions, albeit with a slightly reduced affinity. However, it has lost the ability to resolve these structures by symmetrical cleavage. A multicopy *ruvC51* plasmid confers sensitivity to UV light in a *ruvC*$^+$ strain. The *ruvC53* allele causes a glycine-17 to serine substitution while *ruvC55* produces a stop codon. Neither of these genes produces a stable product. The results suggest that the N-terminal domain of RuvC may be concerned with cleavage of junctions.**

INTRODUCTION

The ability to catalyse recombination between damaged chromosomes and the undamaged regions of homologues plays a vital part in DNA repair. Recombination proceeds via a number of well defined stages in which homologous molecules first pair and exchange strands to produce a symmetrical structure called a Holliday junction (1). Resolution of this junction by specific endonucleolytic cleavage allows segregation of viable (repaired) chromosomes. How junctions are recognized and then resolved is therefore crucial to our understanding of recombination and DNA repair mechanisms (2).

Three distinct activities that resolve junctions have been identified in yeast. The first of these activities cuts 4-way junctions and other branched structures (3), and as such behaves rather like T4 endonuclease VII and T7 endonuclease I (4, 5, 6). These proteins are involved in de-branching bacteriophage DNA prior to packaging (7, 8). The second activity is encoded by *CCE1* (9). The 41 kDa product of this gene cuts inverted repeat structures that mimic some of the features of Holliday junctions by forming cruciforms. Mutants in *CCE1* lack cruciform-cutting activity and show the petite phenotype normally associated with a mitochondrial defect. Mitotic and meiotic levels of recombination are normal. The same activity was identified independently by Symington and Kolodner (10). A third activity was detected in partially purified extracts from cells treated with a DNA-damaging agent (11). In higher organisms, junction cutting activities have been identified in extracts from calf thymus (12), HeLa cells (13), and the human placenta (14).

Recent studies in bacteria have focused on the 19 kDa product of the *ruvC* gene (15, 16, 17, 18). RuvC protein cleaves Holliday junctions made by RecA protein and also synthetic X-junctions that have some homology in the core to allow branch migration, though it will also cut X-junctions that have no homology (17, 18). Mutations in *ruvC* cause sensitivity to DNA damaging agents and also reduce the efficiency of recombination, especially in certain genetic backgrounds (19, 20, 21). Mutations in the *ruvAB* operon located close to *ruvC* result in a very similar phenotype, though unlike *ruvC*, the *ruvAB* operon is regulated by LexA protein and is induced as part of the SOS response to DNA damage (19, 22, 23, 24, 25, 26, 27). Recent studies have shown that the RuvA and RuvB proteins work together to drive branch migration of Holliday junctions in an ATP-dependent reaction (28, 29, 30). However, there is a functional overlap between the *ruv* genes and *recG* and it appears that RecG may also be able to catalyse branch migration (31, 32).

It is clear from the properties of *ruv* and *recG* mutants that the ability to process Holliday junction intermediates is a key stage in recombination and DNA repair. The RuvAB, RuvC and RecG proteins provide new opportunities to investigate how junctions are recognised and resolved. In this paper, we describe a mutation in *ruvC* that affects the RuvC cleavage reaction without eliminating the ability to bind to junction DNA.

MATERIALS AND METHODS

Strains

Escherichia coli K-12 strains N1373 (*ruvC51*), CS85 (*ruvC53*), CS87 (*ruvC55*) are derivatives of AB1157 (F^- Δ(*gpt-proA*)*62 leuB6 ara-14 lacY1 rpsL31 thi-1 his-4 argE3 thr-1 kdgK51*

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rfbD(?) *galK2 xyl-5 mtl-1 tsx-33 supE44*) (33, 34). CS78 is an *eda-51*::Tn*10* derivative of the *ruvC51* strain N1373 (34). JM101 (F128 *proAB*$^+$ *lacI*^{q}*ZΔM15 traD36/endA1 hsdR17 gyrA96 supE44* Δ(*lac-pro*)) (35) was used for generating ssDNA from pGEM-7Zf derivatives. *E.coli* B strain BL21(DE3) (F$^-$ *ompT* $r_B^-m_B^-$) carrying p*lysS* was used to control expression of overproducing plasmids (36). TNM620 is a *ruvC51* derivative of BL21 (DE3) carrying p*lysS*, and was constructed by transduction with P1 phage from CS78 and selecting for resistance to tetracycline (*eda-51*). Since *E.coli* B strains are UV sensitive, inheritance of the linked *ruvC51* was confirmed by backcrossing to strain C600 ($r_K^-m_K^+$ *thi*$^-$ *thr*$^-$ *leu*$^-$ *lacY*$^-$ *hsr*$^-$) (33), which avoids restriction degradation of DNA transduced from *E.coli* B.

Media and general methods

LB broth and agar have been cited, as have P1 transductions and procedures for measuring sensitivity to UV light (19, 21). DNA manipulations followed the recipes and protocols described (37). PCR reactions used *AmpliTaq* polymerase (Perkin Elmer Cetus) and followed standard protocols. Protein gels and autoradiographs were scanned for quantification of the results using a laser densitometer (Molecular Dynamics).

PCR reactions

Mutant *ruvC* alleles were amplified directly from colonies of the relevant strains essentially as described by Krishnan *et al* (38). A large colony was transferred to 50 μl of sterile distilled water in a 1.5 ml Eppendorf tube, vortexed for 3 minutes and incubated at 37°C for 15 minutes. 2 μl of this suspension was added to a standard PCR reaction mixture. Reactions were heated to 94°C for 4 minutes then 30 cycles set up as follows: 1 minute denaturing at 92°C, 1 minute annealing at 50°C and 2 minutes 30 seconds extension at 72°C.

Cloning and DNA sequencing

Two oligonucleotides (5'-GGCCTGCTAGAATTCAAAAAGG-AGGCGCGTGATG-3', 5'-GGAGTGGAAAAGCTTCA-GCCGG-3') were designed with *Eco*RI and *Hin*dIII sites (underlined) for the cloning and subsequent expression of *ruvC*. PCR products amplified from chromosomal DNA were excised from agarose gels and extracted with Geneclean II (Stratech, UK). They were then digested with *Eco*RI and *Hin*dIII and the products ligated into pGEM-7Zf(+) and (−) (Promega) cut with the same enzymes. The *ruvC* plasmids generated were: pGS776 (*ruvC51*), pGS778 (*ruvC53*), pGS780 (*ruvC55*), in pGEM-7Zf(+), and pGS777 (*ruvC51*), pGS779 (*ruvC53*), pGS781 (*ruvC55*), in pGEM-7Zf(−). Nucleotide sequencing was performed by the Sanger dideoxynucleotide chain-termination method (39) using the T7 kit from Pharmacia-LKB Ltd., UK. Single-stranded DNA used as templates for DNA sequencing were obtained from pGEM-7Zf(+/−) recombinants using M13 K07 helper phage and standard protocols (Promega). The sequence of the entire gene was determined for each *ruvC* mutant, using vector primers and two primers (sequence not shown) within *ruvC*.

Overexpression of *ruvC*

The inserts from pGS776, pGS778 and pGS780 were excised with *Eco*RI and *Hin*dIII and recloned into pT7−7 (40) to give pGS784 (*ruvC51*), pGS785 (*ruvC53*), and pGS786 (*ruvC55*), respectively, with *ruvC* expression under the control of the strong T7 φ10 promoter (36, 40). The constructs were then introduced into TNM620 for expression.

Proteins

Wild-type RuvC protein was purified as described (17). RuvC51 was purified from TNM620 carrying pGS784. Four litres of culture were grown to an A_{650} of 0.5 at 37°C in LB broth containing ampicillin (50 μg/ml) and chloramphenicol (25 μg/ml) to maintain selection on the plasmids. Extra ampicillin (40ml of 4 mg/ml stock) was then added to help maintain pGS784 and expression of *ruvC* was induced by adding isopropyl β-D-thiogalactopyranoside (IPTG) to 2 mM and incubating for a further 3 h. Cells were pelleted, resuspended in 32 ml ice-cold 100 mM Tris−HCl pH 8.0, 2 mM EDTA, 5% glycerol, frozen in liquid N_2 and stored at −80°C. The cells were thawed on ice and after adding 0.25 volumes of 5 M NaCl, 0.01 volumes of 0.1 M dithiothreitol (DTT) and 0.01 volumes of 10% Triton X-100, were lysed by three freeze/thaw cycles. The lysate was centrifuged at 40 000 rpm for 1 hour in a Kontron ultracentrifuge using a TST41 rotor, and the less viscous upper half of supernatant was collected (26 ml) and dialysed for 3 hours against buffer A (20 mM Tris−Cl pH 8.0, 1 mM EDTA, 1 mM DTT, 10% glycerol). During lysis of these cells a large proportion of the overproduced RuvC51 precipitated and was located in the cell debris following centrifugation. This substantially reduced the yield of pure protein. The dialysed supernatant was diluted 5-fold in Buffer A, applied to a 60 ml DEAE Biogel A column (BioRad) and developed with a 600 ml 0−500 mM KCl linear gradient in buffer A. Fractions containing RuvC51 were identified by SDS-PAGE (RuvC51 eluted at 200−300 mM KCl), pooled (120 ml, 0.86 mg/ml) and loaded directly onto a 20 ml Reactive Blue 4-Agarose (Sigma) column. Bound proteins were eluted with a 200 ml 0.5−1.25 M linear gradient of KCl in buffer A. RuvC51 eluted in a broad peak between 600 and 800 mM KCl. Pooled fractions (45 ml, 0.16 mg/ml) were dialysed against Buffer A containing 200 mM KCl, loaded onto a 10 ml phosphocellulose column (Whatman P11) and developed with a 100 ml 200−1000 mM linear gradient of KCl in buffer A. RuvC51 eluted at 600 mM KCl. Pooled fractions (17.5 ml, 0.1 mg/ml) were dialysed against buffer A, loaded on a 5 ml ssDNA cellulose (Sigma) column and bound proteins eluted with a 50 ml 0−500 mM gradient of KCl in buffer A. RuvC51 eluted at 300 mM KCl. Fractions containing RuvC51 alone were pooled, dialysed against buffer A containing 50% glycerol and stored in aliquots at −20°C. A total of 0.38 mg of RuvC51 was recovered at greater than 90% purity. The above procedure is adapted from a revised purification scheme for RuvC$^+$ protein (41). The column-binding properties of RuvC51 are much the same as those of the wild-type protein (17, 18, 41). Purified RuvC51 was tested for contaminating exonucleases using the method described in Lovett and Kolodner (45). No exonuclease activity was detectable. Protein concentrations were determined by the Bradford method using a kit from BioRad, with bovine serum albumin (Sigma) as a standard. Protein molecular weight markers were purchased from Sigma.

Synthetic Holliday junctions and duplex DNA

Model Holliday junctions and linear duplex DNA were made by annealing 49−51 mer oligonucleotides as detailed previously (42, 43). Oligonucleotide 1 was labelled with ^{32}P at the 5' end with T4 kinase and [γ^{32}P-ATP] (Amersham) before annealing. Approximately 40 nM of DNA were used in each reaction. DNA concentrations are in moles of nucleotide residues and were determined using DNA dipsticks (Invitrogen, San Diego).

Gel retardation assays

Reaction mixtures (20 μl) contained ^{32}P-labelled synthetic Holliday junction or linear duplex DNA in binding buffer (50 mM Tris−HCl, pH 8.0, 5 mM EDTA, 1 mM DTT, 100 μg/ml bovine serum albumin) and various amounts of the test protein. After 15 min on ice, 5 μl loading buffer (40 mM Tris−Cl, pH 7.5, 4 mM EDTA, 25% glycerol, 400 μg/ml bovine serum albumin) was added and the samples loaded immediately onto 4% polyacrylamide gels in low ionic strength buffer (6.7 mM Tris−Cl, pH 8.0, 3.3 mM sodium acetate, 2 mM EDTA). Electrophoresis was carried out at room temperature for 1.75 h at 200 V with continuous circulation of buffer. Gels were dried on Whatman 3MM paper and subjected to autoradiography.

Cleavage of a synthetic Holliday junction

Reaction mixtures (20 μl) contained synthetic Holliday junction or linear duplex DNA in reaction buffer (50 mM Tris−Cl, pH 8.0, 1 mM DTT, 5 mM $MgCl_2$, 100 μg/ml bovine serum albumin) and various amounts of protein sample. Reactions were incubated at 37°C for 60 min before adding 5 μl stop buffer (2.5% (w/v) SDS, 200 mM EDTA, 10 mg/ml proteinase K) and incubating for a further 10 min at 37°C. The DNA products were then electrophoresed at room temperature through a 10% native polyacrylamide gel using a Tris-borate buffer system (43), followed by autoradiography.

RESULTS

Cloning and nucleotide sequence of *ruvC51, ruvC53 and ruvC55*

Previous studies identified a number of independent mutations in the *ruvC* gene (24, 34, 44). Each of these mutations was cloned using PCR to amplify the *ruvC* gene from colonies of the mutant strains. The method used was designed initially for sequencing plasmid DNA (38), but we found that chromosomal DNA could be amplified easily for cloning. The products amplified were cloned into pGEM-7Zf vectors and sequenced on both strands. Each *ruvC* allele cloned has a single base pair transition. This results in a glycine-15 to aspartic acid substitution in *ruvC51* and glycine-17 to serine substitution in *ruvC53* (Fig. 1A). The *ruvC55* mutation produces a TAG (amber) stop codon at glutamine-60 (Fig. 1B). The G:C to A:T transition in all three cases is consistent with the use of nitrosoguanidine (*ruvC51*) and hydroxylamine (*ruvC53* and *ruvC55*) in the original mutagenesis (34, 44).

The nature of the *ruvC55* mutation was initially surprising, since this allele confers a weak UV sensitivity that is increased by incubation at higher temperatures (34). However, the AB1157 genetic background of the strain used in these studies (CS87) has the amber suppressor, *supE44,* which inserts glutamine at UAG stop codons. Since insertion of glutamine restores the wild-type amino acid sequence, we assume that the moderate UV sensitivity of CS87 is due to reduced efficiency of amino acid insertion by $tRNA_{supE44}$. We also conclude that the temperature sensitivity is caused by the suppressor itself. The *ruvC55* allele confers a typical *ruv* mutant phenotype in the suppressor-free background of strain W3110 (33) (data not shown), while in a *supD* background where a serine is inserted at a UAG codon, it is totally suppressed (data not shown). This observation shows that a glutamine-60 to serine substitution has no effect on the activity of RuvC.

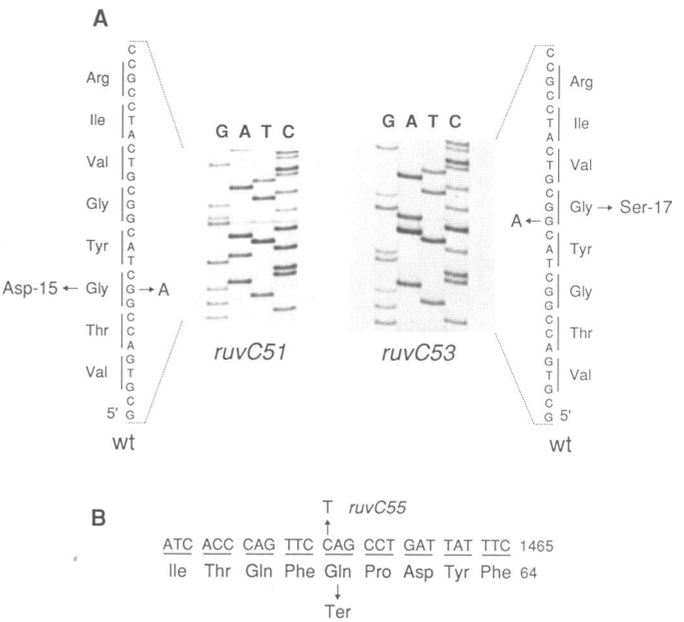

Figure 1. Nucleotide sequence of *ruvC51*, *ruvC53* and *ruvC55* mutations. (**A**) The wild-type *ruvC* DNA and protein are shown beside the *ruvC51* and *ruvC53* sequences. The 5′ end of the nucleotide sequence is position 1308 of the published sequence (16). The base substitutions and resulting amino acid alterations for each mutation are indicated at the side of the wild-type sequence. The amino acids are numbered from the N-terminal methionine although this residue is removed from the protein product. (**B**) The *ruvC55* mutation at amino acid 60 is represented with both wild-type and mutant shown. Position 1465 refers to the published sequence (16). Ter denotes a termination codon.

Effect of the cloned *ruvC* alleles on sensitivity to UV light

The *ruvC* mutant alleles were introduced into strain AB1157 and its *ruv* derivatives, using the plasmid constructs made using pT7-7. The T7 φ10 promoter is inoperative in these strains and any expression of *ruvC* would have to rely on some adventitious promoter. These strains were tested for sensitivity to UV light. The *ruvC51* construct pGS784 made AB1157 quite sensitive to UV despite the presence of the chromosomal ruv^+ allele (Fig. 2). The *ruvC53*, *ruvC55*, and $ruvC^+$ plasmids had no such effect. (Fig. 2 and data not shown). The *ruvC51* and *ruvC53* constructs did not affect survival of N1373, CS85, or CS87, whereas the *ruvC55* construct made all three as resistant as strain AB1157 carrying these plasmids (data not shown). Presumably, the *ruvC55* construct makes enough wild-type RuvC protein to restore UV resistance.

Overproduction of mutant RuvC proteins

The *ruvC* recombinants of pT7-7 were made in such a way that they had an improved ribosome-binding site for expression of *ruvC* from the T7 φ10 promoter. Strain BL21(DE3) p*lysS* carrying these plasmids were grown in LB broth and induced with IPTG. pGS785 (*ruvC53*) and pGS786 (*ruvC55*) failed to produce detectable RuvC proteins (data not shown). pGS784 (*ruvC51*), however, overproduces a protein of about 20 kDa (Fig. 3, lane e). We assume that this is RuvC51 protein due to its absence in uninduced cells (Fig. 3, lane d). Similar amounts of protein (about 10−15% of total cell protein) are produced by an analogous construct (pGS775) carrying the $ruvC^+$ gene (Fig. 3, lane c) (41). To avoid contamination of RuvC51 with

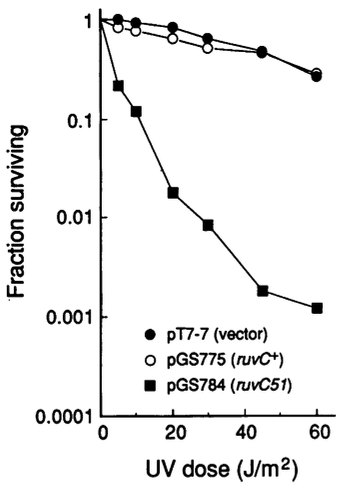

Figure 2. Effect of the cloned *ruvC51* mutant allele on wild-type cells after exposure to UV. pGS775 contains the wild-type *ruvC* gene in pT7-7 and was constructed using the same oligonucleotides used for PCR of the mutant alleles (41). pGS760 (16) DNA was used as a template for PCR rather than colonies. Cells were grown to an A_{650} of 0.4 and appropriate dilutions spotted on plates containing ampicillin (50 μg/ml). All plates were incubated at 37°C for 16 h. UV doses were at a rate of 1 J per m^2 per second.

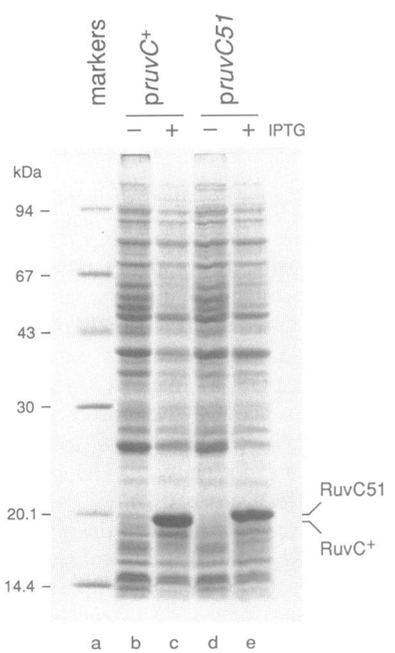

Figure 3. Overproduction of RuvC51 protein. Plus and minus represent the presence or absence of IPTG. Lane a, molecular weight markers; lane b and c, BL21 (DE3) p*lysS* pGS775; lane c and d, BL21 (DE3) p*lysS* pGS784. Strains carrying pGS775 and pGS784 are labelled p*ruvC*$^+$ and p*ruvC51*, respectively.

wild-type RuvC from the *E.coli* B chromosome, a BL21 (DE3) p*lysS ruvC51* derivative was constructed (TNM620) and used as a host for overexpression of RuvC51. RuvC51 protein was purified as described in the Materials and Methods section.

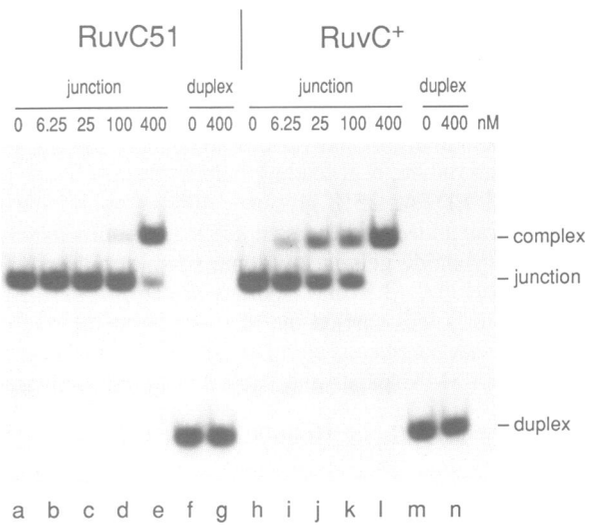

Figure 4. Binding of RuvC51 to synthetic Holliday junctions. Purified RuvC51 and RuvC$^+$ proteins were incubated on ice for 15 minutes with 5′-^{32}P-labelled synthetic Holliday junction (lanes a−e, h−l) or linear duplex DNA (lanes f−g, m−n). Samples were electrophoresed through a 4% low-ionic-strength polyacrylamide gel. Concentrations of RuvC51 and RuvC$^+$ are indicated.

Binding of RuvC51 to synthetic Holliday junctions

RuvC51 protein was examined for its ability to bind to junctions in DNA. We used a small synthetic X-junction containing a 12 bp core of homologous sequences flanked by 18−20 bp of heterologous DNA. This substrate is identical to that used previously (17). Junctions labelled with ^{32}P were mixed with increasing amounts of RuvC51 and RuvC$^+$. Both proteins formed defined complexes with the same mobility. However, with RuvC51 the complex was detected at slightly higher concentrations of protein (Fig. 4, lanes d, e). At 100 nM, RuvC51 bound 8.5% of the junction while RuvC$^+$ bound 40%. At 400 nM, RuvC51 retarded 75%, and RuvC$^+$ 95%. The relatively greater degree of binding seen with RuvC51 at higher concentrations suggests co-operative binding. Under identical conditions, neither protein showed binding to linear duplex DNA (Fig. 4, lanes g and n).

RuvC51 does not cleave the X-junction

RuvC51 clearly retains significant junction-binding activity. To examine its ability to cut the junction, reactions were performed at 37°C in the presence of Mg^{2+}. RuvC$^+$ produced the (nicked) duplex product expected from cleavage of the junction (Fig. 5, lanes i−l). However, we were unable to detect any cutting of the junction with RuvC51 (lanes b−e). In other experiments with different conditions, we used 1, 2 and 4 μM RuvC51, but again we observed no cutting (data not shown). The sensitivity of the assay was such that we would have expected to detect 0.1% of RuvC$^+$ activity.

RuvC51 does not block the resolvase activity of RuvC$^+$

To try and understand why RuvC51 makes a *ruv*$^+$ strain sensitive to UV light, we examined the effect of RuvC51 on the cutting activity of RuvC$^+$. RuvC51 protein was pre-incubated with junction DNA on ice to allow binding. RuvC$^+$ was then added and incubated at 37°C as in the standard cleavage reaction.

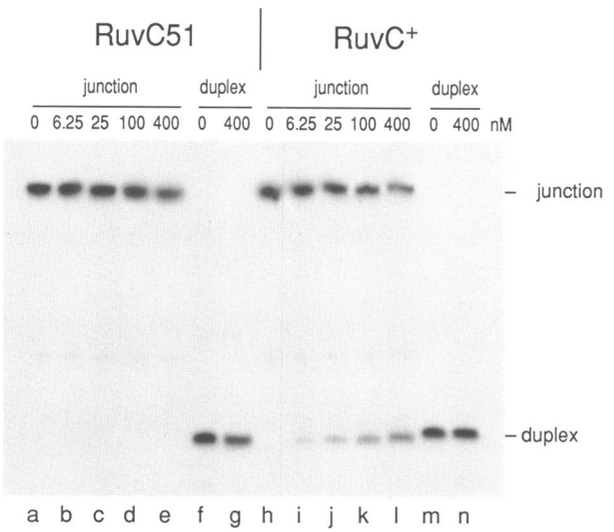

Figure 5. RuvC51 is unable to cleave synthetic Holliday junctions. Purified RuvC51 and RuvC$^+$ proteins were incubated at 37°C for 60 minutes with 5′-^{32}P-labelled synthetic Holliday junction (lanes a–e, h–l) or linear duplex DNA (lanes f–g, m–n). Reactions were terminated by the addition of 5 μl of loading buffer and electrophoresed through a 12% Tris-borate polyacrylamide gel. Concentrations of RuvC51 and RuvC$^+$ are indicated.

RuvC51 did not inhibit the RuvC$^+$ cleavage reaction even up to 160 times the concentration of RuvC$^+$ (data not shown). In a similar experiment the two proteins were mixed and incubated on ice for 30 minutes before adding to the reaction mixture. Again, the cutting activity was not affected (data not shown).

DISCUSSION

We have described the cloning and characterization of three *ruvC* mutations and identified a mutant protein, RuvC51, that binds X-junctions but has lost the ability to resolve these into duplex products. RuvC51 behaved very much like RuvC$^+$ during purification, with the exception that more of the RuvC51 precipitated during lysis. The precipitated RuvC51 could not be recovered from the pellet using sonication and/or high salt. Lysis procedures using DNase I to remove DNA also resulted in precipitation of RuvC51. RuvC is thought to function as a dimer (18). When separated by SDS-PAGE without reducing agent or boiling RuvC51 produced faint higher molecular weight bands that appeared to correspond to dimers, trimers and tetramers. RuvC$^+$ under the same conditions gave a band of about 40 kDa, presumably a dimer (data not shown). Purified RuvC51 did produce the same size of complex with synthetic Holliday junction DNA as RuvC$^+$. However, it is possible that RuvC51 can multimerize more easily than RuvC$^+$ producing the reduced binding affinity for junctions and explaining the greater tendency of RuvC51 to precipitate. RuvC51 migrates in SDS-PAGE as a 20kDa protein, rather than the 19 kDa of RuvC$^+$. This is probably due to an increase in overall negative charge. The RuvC51 protein has a predicted charge at pH 7 of 5.36 whereas RuvC$^+$ has a charge of 6.36. These values are calculated after removal of the N-terminal methionine which occurs *in vivo* (17, 18).

The cloned *ruvC51* allele, but not *ruvC*$^+$, was found to make a *ruv*$^+$ strain sensitive to UV light. We attempted to mimic this effect *in vitro* by including RuvC51 in reactions containing RuvC$^+$ and synthetic Holliday junctions. RuvC51 failed to reduce RuvC$^+$ cleavage even when present in 160-fold excess. The *in vivo* observations may be due to even higher levels of RuvC51 expressed from the plasmid relative to the amount of RuvC$^+$ from the chromosome. From our understanding of the expression of *ruvC* this explanation is quite reasonable. Another possibility is that RuvC51 is able to form inactive heterodimers with RuvC$^+$. This would be difficult to test *in vitro* if dimerisation has already taken place in the separately purified samples. It may be that the RuvC51 protein is only partially folded in our purified samples. This could produce a more extreme effect on the junction-cleavage reaction than on its ability to bind DNA. Another *ruvC* mutation has recently been obtained in pGS775. This has an aspartic acid-8 to asparagine substitution which results in a defective protein *in vivo*. This mutant plasmid, like pGS784, produces a negative effect on AB1157 following exposure to UV (N.F.Hagan, G.J.Sharples and R.G.Lloyd, unpublished results).

The *ruvC55* mutation results in termination of translation near the middle of the gene. This amber mutation has been used to insert a serine at position 60 in place of glutamine, using the *supD* suppressor. This alteration had no effect on the ability of the gene product to confer resistance to UV light, which suggests that this position is not so vital for activity. RuvC53 (gly-17 to ser) failed to overproduce a protein product. This is most likely due to the inability to fold correctly, leading to degradation by cytoplasmic proteases.

In conclusion, it is tempting to speculate that the N-terminal β-sheet portion of RuvC functions in the cutting reaction. The RuvC51 mutation could either affect correct folding of the protein in this region or directly disrupt the active site in a more localized manner. The C-terminal region of RuvC from residue 63 has several predicted α-helices. In the HMG1 protein of rat it is the positioning of α-helices in the HMG box motif that has been linked with the ability of HMG1 to bind X-junctions (46).

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