A mutation in helicase motif III of *E.coli* RecG protein abolishes branch migration of Holliday junctions

Gary J.Sharples, Matthew C.Whitby, Lizanne Ryder and Robert G.Lloyd*

Department of Genetics, University of Nottingham, Queens Medical Centre, Nottingham NG7 2UH, UK

Received November 2, 1993; Revised and Accepted December 21, 1993

ABSTRACT

The RecG protein of *Escherichia coli* catalyses branch migration of Holliday junctions made by RecA and dissociates synthetic X junctions into duplex products in reactions that require hydrolysis of ATP. To investigate the mode of action of this enzyme a chromosomal mutation that inactivates *recG* (*recG162*) was cloned and sequenced. The *recG162* mutation is a G:C to A:T transition, which produces an Ala428 to Val substitution in the protein. This change affects a motif (motif III) in the protein that is highly conserved in DNA and RNA helicases. RecG162 protein was purified and shown to retain the ability to bind synthetic X and Y junctions. However, it does not dissociate these junctions and fails to catalyse branch migration of Holliday junction intermediates purified from a RecA strand exchange reaction. RecG162 retains a DNA-dependent ATPase activity, but this is much reduced relative to the wild-type protein, especially with single-stranded DNA as a co-factor. These results suggest that branch migration by RecG is related to a junction-targeted DNA helicase activity.

INTRODUCTION

The formation and resolution of Holliday junctions are key stages in homologous recombination and DNA repair. Holliday junctions are the intermediates formed during recombination when homologous DNA molecules pair and exchange strands (1). In *Escherichia coli*, these intermediates are formed by RecA protein which polymerises on single-stranded DNA to form a helical RecA–DNA filament that initiates a search for homologous sequences. Once homologous contacts have been established, RecA promotes pairing of the DNA and initiates the exchange of single strands (2). In duplex–duplex interactions, a reciprocal exchange of strands leads directly to a Holliday junction that is then processed into recombinant products by other proteins. Four *E.coli* proteins have been shown to act on these junctions *in vitro*. These are the products of the *ruvA*, *ruvB*, *ruvC* and *recG* genes, all four of which are needed for normal recombination and DNA repair (3–8). RuvA protein binds to DNA and forms specific complexes with synthetic X junctions (9, 10), while RuvB is a

weak ATPase. These two proteins act together to catalyse branch migration of Holliday junctions made by RecA. They also dissociate synthetic X junctions into linear, partially duplex products (9–11). The reaction mechanism is not fully understood, but probably involves a specialised helicase activity (12). RuvC protein is a nuclease. It interacts specifically with Holliday junctions and resolves these into recombinant products by symmetrical cleavage across the point of strand exchange (13–15).

The 76 kDa RecG protein has features in common with both RuvA and RuvB. It binds DNA, is a potent ATPase, and like RuvAB is able to dissociate synthetic X junctions to duplex products (16). It can also drive branch migration of Holliday junctions made by RecA (17). The similar activities of RuvAB and RecG fit with the synergistic interactions observed when mutations in *ruv* and *recG* are combined (18). Presumably, RuvAB and RecG can to some extent substitute for one another *in vivo*. The precise situation remains unclear since the synergistic interaction extends to *ruvC* (18). There is no indication that RecG is able to cleave junctions (16). A possible explanation for this apparent inconsistency is a model that proposes that RecG can resolve Holliday junctions by a reverse branch migration process (17). Another possibility is that RecG works with a second Holliday junction resolvase. A mutation at the *rus* locus that suppresses the phenotype of *ruv* mutations is a candidate for such an activity (19).

The amino acid sequence of RecG shows all seven of the motifs generally conserved in DNA and RNA helicases (20, 21). In this paper we describe a mutation in *recG* that affects motif III. The properties of the mutant protein suggest that branch migration of Holliday junctions may be a specialised form of DNA helicase activity.

MATERIAL AND METHODS

Strains

GS1451 is an *E.coli* B strain BL21(DE3) *physS* (22, 23) carrying $\Delta recG263 :: kan$ (formerly $\Delta spoVI$) transduced in with a P1 phage from CF3324 (24). N2731 (*recG258*) is a derivative of the *E.coli* K-12 strain AB1157 (8, 25). PC0297 carries *recG162* (7). JM101 was used to prepare ssDNA for sequencing (26).

*To whom correspondence should be addressed

Construction of a plasmid for expression of *recG162*

The *recG* locus of strain PC0297 was amplified by PCR using single colonies (27). The oligonucleotides, 5'-GCAGGCTGC-AGGGTAAGTCATATGAAAGGT-3' and 5'-CATCCGGCAGGAAGCTTGGGTAACCTGAAA-3' were used as primers to incorporate *NdeI* and *HindIII* sites (underlined) at the 5' and 3' ends, respectively. The PCR product was cleaved with *NdeI* and *HindIII* and ligated into similarly digested pT7-7 to give pGS809, which places *recG162* expression under control of the T7 phage $\phi 10$ promoter (28).

DNA sequencing

The *recG162* allele was subcloned from pGS809 into pGEM-7Zf (+) and (-) (Promega) to provide single-stranded template DNA. Sequencing was by the dideoxynucleotide chain-termination method (29) using a T7 polymerase sequencing kit (Pharmacia) and primers corresponding to vector or *recG* sequences.

DNA substrates

Synthetic X junction, Y junction and linear duplex DNA substrates were made by annealing synthetic 49–51-mer oligonucleotides (30). The numbering and sequence of the oligonucleotides used have been described (13, 14, 16, 31). In all cases, oligonucleotide 1 was ^{32}P -end-labelled at the 5' end before annealing. Junction DNA was measured using DNA DipSticks (Invitrogen, San Diego), and is approximate due to the low concentration. Circular ϕX174 viral (+) strand and RF DNA were from Pharmacia-LKB. Gapped circular DNA and *PstI*-linearized duplex DNA were made from the RF DNA as described (32). The linear duplex was labelled at the 3' end using terminal transferase and $[\alpha\text{-}^{32}\text{P}]\text{ddATP}$. DNA concentrations are in nucleotide equivalents.

Proteins

The RecA used was a gift from S.C. West (32). Wild-type RecG was purified essentially as described (16) except that for the final step in the fractionation a 1 ml Mono Q column was used in place of hydroxylapatite. The running of this column was similar to that described for RecG162 (below). RecG⁺ eluted from the Mono Q at approximately 220 mM KCl. A total of 7.6 mg of RecG⁺ was purified at 1.4 mg/ml from 6 l of cells. RecG162 was purified from a 4 litre batch of strain GS1451 carrying pGS809 as for RecG⁺ (16) with the following modifications (RecG162 was monitored throughout by SDS-PAGE). The supernatant from the cell lysate was dialysed for 3.5 h against buffer A [20 mM Tris-HCl, pH 8.5, 1 mM EDTA, 0.5 mM dithiothreitol (DTT), 10% glycerol] containing 0.5 mM phenylmethylsulphonyl fluoride (PMSF), loaded onto a 70 ml DEAE BioGel A (BioRad) column, and bound proteins eluted with 350 ml of a 0–600 mM gradient of KCl in the same buffer. RecG162 fractions eluting at 250 mM KCl were pooled (38 ml, 8.75 mg/ml protein), dialysed against buffer A pH 7.5 and loaded onto a 15 ml ssDNA cellulose (Sigma) column. Bound proteins were eluted with a 150 ml linear gradient of 0–1000 mM KCl in buffer A pH 7.5. RecG162 eluted at 300 mM KCl and the peak fractions were pooled (70 ml, 0.15 mg/ml protein), dialysed against buffer A pH 7.5 and loaded onto a 1 ml Mono Q column (Pharmacia). Bound proteins were eluted with a 25 ml linear gradient of 0–500 mM KCl. Fractions containing RecG162 alone eluted between 200 and 250 mM KCl, and were pooled (2 ml,

2.5 mg/ml protein) and dialysed against buffer A pH 7.5. To remove a contaminating exonuclease remaining at this stage, a sample of the Mono Q fraction (0.7 mg protein) was applied to a Superose 12 gel filtration column (Pharmacia-LKB) using buffer A pH 7.5, 150 mM KCl. The peak fractions of RecG162 were free of nuclease activity. These were pooled, mixed with an equal volume of 100% glycerol, and stored in aliquots at -80°C (1.5 ml, 0.4 mg/ml protein). Exonuclease activity was determined using ^{32}P -labelled λ DNA (33). Protein concentrations were determined by the Bradford method using a kit from BioRad and bovine serum albumin (Sigma) as a standard.

ATPase assay

The release of $[\alpha\text{-}^{32}\text{P}]$ ADP from $[\alpha\text{-}^{32}\text{P}]$ ATP was measured by thin layer chromatography on PEI-cellulose (16).

DNA binding assays

Binding of RecG proteins to ^{32}P -labelled synthetic X and Y junctions and linear duplex DNA was detected using a gel retardation assay (16, 31). Filter binding assays were conducted essentially as described by McEntee *et al.* (34). Binding reactions (20 μl) were performed in 20 mM Tris-HCl pH 7.5, 5 mM MgCl_2 , 2 mM DTT, 100 $\mu\text{g/ml}$ BSA, using 45 ng *PstI*-linearized ϕX174 RF DNA with (ssDNA) or without (dsDNA) heat denaturation, and 0.95 μM RecG or RecG162.

Dissociation of synthetic junctions

The dissociation of ^{32}P -labelled synthetic X junction DNA into linear duplex products with unpaired single-strand ends was measured as described (16). The two products observed correspond to oligonucleotides 1+2 and 1+4. Dissociation of a ^{32}P -labelled synthetic Y junction was measured in a similar manner. The product of this reaction is a single band corresponding to oligonucleotides 1+4 (31).

Formation, deproteinization and dissociation of Holliday intermediates made by RecA

A standard RecA reaction was used to catalyse strand exchange between gapped circular duplex DNA and ^{32}P -labelled linear duplex DNA as described (35). The reactions were deproteinized and Holliday junction intermediates purified as described (35). Dissociation of the purified intermediates was conducted in buffer containing 20 mM Tris-HCl pH 7.5, 5 mM MgCl_2 , 2 mM DTT, 100 $\mu\text{g/ml}$ BSA, 20 mM phosphocreatine, 12.5 units/ml creatine phosphokinase, 2 mM ATP, and protein as indicated. Reactions were deproteinized, and the products analysed on 0.8% agarose gels using a Tris-acetate buffer system (40 mM Tris-acetate pH 8.0, 1 mM EDTA) at 6 V for 2.5 h. Gels were dried and the labelled products visualised by autoradiography.

RESULTS

Nucleotide sequence of *recG162*

The *recG* locus was first identified in strain PC0297 as a nitrosoguanidine-induced mutation that conferred a modest sensitivity to UV light (7). The *recG162* mutation was amplified by PCR from this strain and cloned into a plasmid vector. Nucleotide sequencing of the entire gene revealed a single change in the coding region, a G:C to A:T transition that causes an Ala428 to Val substitution in the RecG polypeptide (Fig. 1A). This change affects motif III (Fig. 1B), one of the seven motifs generally conserved in DNA and RNA helicases (21). To confirm

Figure 1. A. Sequence analysis showing the G:C to A:T transition in *recG162* and the resulting amino acid substitution. The numbers relate to the published sequence (20). **B.** Alignment of motif III of RecG162, p68 (51), eIF-4A (52), PRP22 (38), ERCC3 (53), ERCC6 (44) and RAD54 (42). The boxed regions are based on the consensus for motif III (21). The black shaded background denotes identity with the S/T, A/G, S/T consensus and grey shading the conserved hydrophobic residues (+), V, I, L and M preceding this region.

that the mutation was not generated during the PCR reaction, a further three plasmid isolates were sequenced using a primer homologous to this region. All of these plasmids carry the same transition mutation at base pair 1709.

Purification of RecG162

For purification of RecG162 we used a strain (GS1451) deleted for the chromosomal *recG* locus to avoid any possible contamination with RecG⁺ and introduced *recG162* on a plasmid construct (pGS809) under the control of the ϕ 10 promoter of phage T7. When this promoter is activated by the addition of isopropyl β -D-thiogalactopyranoside (IPTG), a protein of 76 kDa accumulates to some 5–10% of the total cell protein within 3 h at 37°C (data not shown). We assume this is RecG162 since a protein of identical mobility as judged by SDS–PAGE was induced by the analogous construct carrying the *recG⁺* gene (data not shown). RecG162 was purified by a modification of the procedure described for the wild-type RecG (16). Unlike the wild-type, RecG162 binds to DEAE BioGel A, which reduced the purification achieved at this stage. The final fraction was estimated to be >95% RecG162 as determined by densitometry (Fig. 2, lane c). Analysis of the gel filtration used as the final step in the purification indicated that RecG162 is a monomer in solution, as is RecG⁺ (data not shown), though it probably functions as a multimer on junction DNA (see below).

ATPase activities of RecG162

RecG has been shown to be a very potent DNA-dependent ATPase. RecG162 is also a strong ATPase, but its activity is reduced significantly relative to the wild-type protein (Fig. 3). The reduction is particularly noticeable with single-stranded DNA (ssDNA) as a cofactor, with only 121 nM of ATP converted to ADP after 90 min (Fig. 3A), compared with 491 nM in the presence of double-stranded DNA (dsDNA) (Fig. 3B). RecG has some ATPase activity in the absence of DNA. The amount of ATP

Figure 2. SDS–PAGE analysis of purified RecG⁺ and RecG162. Lane a, markers; lane b, 5 mg RecG⁺; lane c, 5 mg RecG162. Proteins were resolved on a 12.5% gel and stained with Coomassie brilliant blue.

Figure 3. ATPase activities of RecG proteins. Reaction mixtures (100 μ l) contained 50 mM Tris–HCl, pH 7.5, 5 mM MgCl₂, 1 mM DTT, 1 mM ATP, and 0.01–0.05 μ Ci [α -³²P] ATP, 0.2 μ M protein, and either 22 μ M ssDNA (ϕ X174 + strand) (A) or 30 μ M supercoiled RF DNA (B). Incubation was at 37°C and 10 μ l samples removed as indicated and quenched with 2.5 μ l of 0.5 M EDTA before separation on PEI–cellulose.

hydrolysed within 90 min without DNA was 163 nM for RecG⁺ and 6 nM for RecG162 (data not shown).

Binding of RecG162 to DNA

Previous studies showed that RecG makes specific complexes with a synthetic X junction (16). We suspected that RecG162 retains the ability to bind DNA from its affinity for ssDNA cellulose (see purification). This was confirmed using a filter-binding assay. RecG⁺ and RecG162 bound 47.0% and 46.8% of the input linear ssDNA, respectively. RecG⁺ binds very

Figure 4. Gel retardation assay showing binding of RecG proteins to a synthetic X junction. Reaction mixtures (20 μ l) contained 32 P-labelled synthetic junction or linear duplex DNA (~ 0.5 μ M) in binding buffer (50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 1 mM DTT, 100 μ g/ml bovine serum albumin) and protein as indicated. After 15 min on ice, 5 μ l loading buffer (40 mM Tris-HCl, pH 7.5, 4 mM EDTA, 25% glycerol, 400 μ g/ml bovine serum albumin) was added and the samples loaded immediately onto 4% polyacrylamide gels in low ionic strength buffer (6.7 mM Tris-HCl, pH 8.0, 3.3 mM sodium acetate, 2 mM EDTA). Electrophoresis was carried out at room temperature for 1.75 h at 160 V with continuous circulation of buffer. Gels were dried on Whatman 3MM paper and exposed to X-ray film.

poorly to linear dsDNA and only 4% of the substrate was retained on the filter under the conditions used. A similar low level of binding was observed with RecG162 (data not shown).

We used a simple band-shift assay to show that RecG162 is able to form specific complexes with a synthetic X junction (Fig. 4, lanes f–h). By comparison with RecG⁺ (lanes b–d), its affinity for the junction seems unaltered and the two complexes formed in each case have identical mobilities. The slower-migrating complex probably contains a dimer or higher multimer of RecG as discussed previously (31). Neither protein showed any binding to a duplex linear control at the concentration used (lanes j and k). The junction used contains a homologous core that is free to branch migrate flanked by heterologous sequences in the arms to prevent dissociation of the structure spontaneous branch migration to the ends (13). However, the homology is not essential for binding since RecG also forms specific complexes with a static Y junction (31). The same is true for RecG162 (Fig. 5). We conclude that RecG162 retains the ability to bind junction DNA with the same specificity as the wild-type protein.

The band-shift assays described in Figures 4 and 5 were conducted in the absence of divalent cations. To compare RecG⁺ and RecG162 under more physiological conditions, we repeated the assays with the X junction using buffers containing either 5 mM MgCl₂ or 5 mM MgCl₂ and 1 mM ATP- γ -S. The complexes formed were less stable under these conditions, but no differences were observed between the two proteins (data not shown).

Figure 5. Binding of RecG proteins to a synthetic Y junction. Assays were conducted as in the legend to Fig. 4, except that Y-DNA (~ 0.4 μ M) replaced the X junction. Binding was quantified by laser densitometry (Molecular Dynamics) of the X-ray films.

Figure 6. Assay for dissociation of a synthetic X junction by RecG proteins. Reaction mixtures (20 μ l) contained 32 P-labelled synthetic X junction and protein as indicated. Reactions were incubated at 37°C for 30 min before adding 5 μ l 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 (30), followed by autoradiography.

Dissociation of synthetic junctions

In the presence of ATP and Mg²⁺, RecG protein dissociates the synthetic X junction described into duplex products with unpaired single-strand ends corresponding to the heterologous arms (16). We were unable to detect dissociation of the junction with RecG162 protein (Fig. 6, lanes g–j). In other experiments (data not shown) densitometric tracings of the X-ray films revealed less than 0.1% of the activity of the wild-type protein. RecG⁺ can also dissociate a Y junction, a feature that is taken as evidence of a DNA helicase activity (31). We examined the effect of RecG162 on this substrate. Less than 0.5% of the wild-type level of dissociation was observed (data not shown).

Branch migration of Holliday intermediates

Although the synthetic X junction provides a close mimic of a Holliday intermediate, it lacks the full symmetry of a true Holliday junction. We therefore used a standard RecA reaction to generate Holliday intermediates between gapped circular duplex DNA and linear duplex DNA. These intermediates were

Figure 7. Assay for branch migration of Holliday intermediates. Reaction mixtures (20 μ l) contained 4.7 μ M deproteinized DNA from a RecA strand exchange reaction (lane a) and 200 nM RecG⁺ (lane b) or 460 nM RecG162 (lane c). Reactions were incubated for 15 min, deproteinized, and the products separated on a 0.8% agarose gel.

deproteinized and used in further reactions with the RecG proteins. With RecG⁺ the intermediates disappeared rapidly while the radioactivity in the DNA band migrating in the position of a nicked circular duplex increased substantially (Fig. 7, lane b). The reaction required ATP (data not shown). The nicked circular DNA corresponds to one of the two products expected from branch migration of the intermediates, the other being the gapped duplex. The formation of the latter is not detected in this reaction since it co-migrates with the much more intense band of linear duplex substrate (32). The nicked circular duplex was not produced by RecG162 (Fig. 7, lane c). We conclude that RecG162 has lost the ability to catalyse branch migration.

A multicopy *recG162* plasmid confers sensitivity to UV light

The *recG162* plasmid construct pGS809 was introduced into N2731 (*recG258*) and AB1157 (*recG*⁺) to see how it affected sensitivity to UV light. The T7 ϕ 10 promoter of the plasmid is inoperative in these strains and any expression of *recG162* would have to come from vector sequences with adventitious promoter activity. pGS809 caused a slight increase in UV sensitivity in N2731 compared with the same strain carrying the vector control (Fig. 8A). However, it made the *recG*⁺ strain AB1157 as sensitive to UV light as N2731 (8) (Fig. 8B). The *recG*⁺ plasmid construct did not have this effect, from which we deduce that RecG162 must be expressed well enough from pGS809 to interfere with the activity of the wild-type RecG encoded by the chromosome.

DNA helicases generally function as dimers, or higher multimers (36). We examined the possibility that RecG162 might poison RecG⁺ by forming inactive multimers, or by blocking the junction. Protein mixtures containing 20 nM RecG⁺ and 7–2000 nM RecG162 were therefore examined for their ability to dissociate the X junction under standard assay conditions. Some inhibition of dissociation was detected, although the effect was slight and 70% of the activity remained even when RecG162 was

Figure 8. Effect of the cloned *recG162* gene on *recG258* mutant (A) and wild-type (B) strains after exposure to UV. pGS772 carries the *recG*⁺ gene in pT7-7 (16). Cells were grown to an OD₆₅₀ of 0.4 and appropriate dilutions irradiated on agar plates containing ampicillin (50 μ g/ml). Colonies of survivors were scored after 16 h at 37°C. UV irradiation was at a dose rate of 1 J/m²/s. Plasmids are identified within panel A.

in 100-fold excess (data not shown). In another experiment RecG162 was mixed with the X junction prior to the addition of RecG⁺ and then incubated for a further 15 min at 37°C. Again the reduction in dissociation was only 3-fold when RecG162 was in 128-fold excess (data not shown). These levels of inhibition do not seem sufficient to account for the negative effect of RecG162 *in vivo*. We cannot rule out the possibility that pGS809 may increase the ratio of RecG162 to RecG⁺ by much more than the maximum used in the dissociation assays. Alternatively, the instability of RecG–junction complexes (Fig. 4) (16, 31), might tend to favour dissociation by RecG⁺ even when RecG162 is present in considerable excess. The situation *in vivo* could be very different, with other proteins available to make more stable complexes.

DISCUSSION

The RecG protein of *E. coli*, like RuvAB, defines a novel activity that catalyses branch migration of Holliday junction intermediates in recombination. In this paper we describe a mutant RecG protein that retains the ability to bind DNA and form specific complexes with synthetic X and Y junctions, but which has lost the ability to dissociate these junctions or to catalyse branch migration of Holliday intermediates made by RecA. RecG belongs to a subsection of DNA/RNA helicase superfamily II (21). This subsection is characterised by seven conserved motifs, with motif II (DExH) as the specialised B region for ATP binding (37). Members of this family are involved in mRNA splicing (38), transcription regulation (39, 40), chromosome stability (41), and DNA repair (42–44). The mutation in RecG162 replaces a highly conserved alanine residue in motif III with a valine. In the RNA helicase eIF-4A, changing Ser–Ala–Thr in motif III to Ala–Ala–Ala eliminates all RNA helicase activity (45). The Thr–Ala–Thr to Thr–Val–Thr alteration in RecG162 results in a loss of all junction dissociation and branch migration activity. The results indicate that RecG catalyses branch migration of Holliday junctions by a DNA helicase-type mechanism that causes localised melting of base pairs at the junction.

Recent studies have shown that RuvAB has a DNA helicase activity that is able to remove a single strand fragment annealed

to a longer section of single-stranded DNA. RecG appears to have no such activity, at least with annealed fragments of 52 and 182 nucleotides (31). However, the fact that it melts the heterologous arms of a synthetic X junction and dissociates a Y junction (31) clearly suggests helicase activity. Presumably, RecG requires a specialised structure such as a junction to initiate this function. The inability to unwind a more conventional helicase substrate is shared with TRCF (Mfd protein), which directs strand-specific excision repair of UV-damaged DNA (46, 47). These two proteins have substantial homology in the region of the helicase motifs (38% identity over 387 amino acids) and may define a new subclass of DNA/RNA helicases that require a specific structure to initiate unwinding. DNA helicases function as dimers or higher multimers. Gel filtration of RecG suggests that the protein is a monomer in solution. However, it probably forms dimers or multimers on substrate (junction) DNA as in the case of Rep (48). This would be consistent with the two defined complexes observed when RecG is mixed with junction DNA.

RecG cleaves ATP to ADP more rapidly with supercoiled replicative form (RF) DNA as a cofactor than with ssDNA (16). This difference is even greater with RecG162, which has reduced ATPase activity overall. The reason for the effect of DNA conformation is unclear, but it may be due to the ability of secondary structures in the supercoiled RF molecule to improve RecG binding. A correlation between branch migration and ATP hydrolysis would be expected if RecG melts duplexes by translocating along the DNA.

In conclusion, the data presented suggests that branch migration of Holliday junctions is probably a modified or specialised form of DNA helicase activity. Holliday junctions are more stable than previously thought and do not spontaneously branch migrate at a high rate (35, 49). They are dissociated rapidly by both RecG and RuvAB (11). Presumably the helicase activity of these proteins drives along two homologous arms of the junction to overcome any energetic barriers to migration, especially mismatches that might otherwise limit the formation of heteroduplex DNA (50).

ACKNOWLEDGEMENTS

We would like to thank Lynda Harris, Carol Buckman and Lisa Corbett for technical assistance. We also thank Steve West for the gift of RecA protein and Stuart Levett for some plasmid substrates. Oligonucleotides were obtained from the Department of Biochemistry, University of Nottingham, UK. This work was supported by the Medical Research Council, Science and Engineering Research Council, the Royal Society and The Wellcome Trust.

REFERENCES

- Holliday, R. (1964) *Genet. Res. Camb.* **5**, 282–304.
- West, S. C. (1992) *Ann. Rev. Biochem.* **61**, 603–640.
- Otsuji, N., Iyehara, H. and Hideshima, Y. (1974) *J. Bacteriol.* **117**, 337–344.
- Lloyd, R. G., Benson, F. E. and Shurvinton, C. E. (1984) *Mol. Gen. Genet.* **194**, 303–309.
- Iwasaki, H., Shiba, T., Nakata, A. and Shinagawa, H. (1989) *Mol. Gen. Genet.* **219**, 328–331.
- Sargentini, N. J. and Smith, K. C. (1989) *Mutat. Res.* **215**, 115–129.
- Storm, P. K., Hoekstra, W. P. M., De Haan, P. G. and Verhoef, C. (1971) *Mutat. Res.* **13**, 9–17.
- Lloyd, R. G. and Buckman, C. (1991) *J. Bacteriol.* **173**, 1004–1011.
- Iwasaki, H., Takahagi, M., Nakata, A. and Shinagawa, H. (1992) *Genes Dev.* **6**, 2214–2220.
- Parsons, C. A., Tsaneva, I., Lloyd, R. G. and West, S. C. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5452–5456.
- Tsaneva, I. R., Müller, B. and West, S. C. (1992) *Cell* **69**, 1171–1180.
- Tsaneva, I. R., Müller, B. and West, S. C. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 1315–1319.
- Connolly, B., Parsons, C., Benson, F. E., Dunderdale, H. J., Sharples, G. J., Lloyd, R. G. and West, S. C. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6063–6067.
- Dunderdale, H. J., Benson, F. E., Parsons, C. A., Sharples, G. J., Lloyd, R. G. and West, S. C. (1991) *Nature* **354**, 506–510.
- Iwasaki, H., Takahagi, M., Shiba, T., Nakata, A. and Shinagawa, H. (1991) *EMBO J.* **10**, 4381–4389.
- Lloyd, R. G. and Sharples, G. J. (1993) *EMBO J.* **12**, 17–22.
- Whitby, M. C., Ryder, L. and Lloyd, R. G. (1993) *Cell* **75**, 1–10.
- Lloyd, R. G. (1991) *J. Bacteriol.* **173**, 5414–5418.
- Mandal, T., Mahdi, A. A., Sharples, G. J. and Lloyd, R. G. (1993) *J. Bacteriol.* **175**, 4325–4334.
- Lloyd, R. G. and Sharples, G. J. (1991) *J. Bacteriol.* **173**, 6837–6843.
- Gorbalenya, A. E., Koonin, E. V., Donchenko, A. and Blinov, V. M. (1989) *Nucleic Acids Res.* **17**, 4713–4730.
- Studier, F. W. and Moffat, B. (1986) *J. Mol. Biol.* **189**, 113–130.
- Studier, F. W., Rosenberg, A. H. and Dunn, J. J. (1991) *Methods Enzymol.* **185**, 60–89.
- Kalman, M., Murphy, H. and Cashel, M. (1992) *Gene* **110**, 95–99.
- Bachmann, B. J. (1987) In Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M. and Umberger, H. E. (eds), *Escherichia coli and Salmonella typhimurium Cellular and Molecular Biology*. American Society for Microbiology, Washington, DC, pp. 1190–1219.
- Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene* **33**, 103–119.
- Krishnan, B. R., Blakesley, R. W. and Berg, D. E. (1991) *Nucleic Acids Res.* **19**, 1153.
- Tabor, S. and Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1074–1078.
- Sanger, F., Nicklen, S. and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
- Parsons, C. A., Kemper, B. and West, S. C. (1990) *J. Biol. Chem.* **265**, 9285–9289.
- Lloyd, R. G. and Sharples, G. J. (1993) *Nucleic Acids Res.* **21**, 1719–1725.
- Müller, B., Jones, C., Kemper, B. and West, S. C. (1990) *Cell* **60**, 329–336.
- Lovett, S. T. and Kolodner, R. D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2627–2631.
- McEntee, K., Weinstock, G. M. and Lehman, I. R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 857–861.
- Müller, B., Burdett, I. and West, S. C. (1992) *EMBO J.* **11**, 2685–2693.
- Matson, S. W. and Kaiser-Rogers, K. A. (1990) *Ann. Rev. Biochem.* **59**, 289–329.
- Koonin, E. V., Choi, G. H., Nuss, D. L., Shapira, R. and Carrington, J. C. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10647–10651.
- Company, M., Arenas, J. and Abelson, J. (1991) *Nature* **349**, 487–493.
- Davis, J. L., Kunisawa, R. and Thorner, J. (1992) *Mol. Cell. Biol.* **12**, 1879–1892.
- Laurent, B. C., Treitel, M. A. and Carlson, M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2687–2691.
- Girdham, C. H. and Glover, D. M. (1991) *Genes Dev.* **5**, 1786–1799.
- Emery, H. S., Schild, D. S., Kellog, D. E. and Mortimer, R. K. (1991) *Gene* **104**, 103–106.
- Schild, D., Glassner, B. J., Mortimer, R. K., Carlson, M. and Laurent, B. C. (1992) *Yeast* **8**, 385–395.
- Troelstra, C., van Gool, A., de Wit, J., Vermeulen, W., Bootsma, D. and Hoeijmakers, J. H. J. (1992) *Cell* **71**, 939–953.
- Pause, A. and Sonenberg, N. (1992) *EMBO J.* **11**, 2643–2654.
- Selby, C. R., Witkin, E. M. and Sancar, A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11574–11578.
- Selby, C. P. and Sancar, A. (1993) *Science* **260**, 53–58.
- Lohman, T. M. (1993) *J. Biol. Chem.* **268**, 2269–2272.
- Johnson, R. D. and Symington, L. S. (1993) *J. Mol. Biol.* **229**, 812–820.
- Panyutin, I. G. and Hsieh, P. (1993) *J. Mol. Biol.* **230**, 413–424.
- Ford, M. J., Anton, I. A. and Lane, D. P. (1988) *Nature* **332**, 736–738.
- Nielsen, P. J., McMaster, G. K. and Trachsel, H. (1985) *Nucleic Acids Res.* **13**, 6867–6880.
- Weeda, G., van Ham, R. C. A., Vermeulen, W., Bootsma, D., van der Eb, A. J. and Hoeijmakers, J. H. J. (1990) *Cell* **62**, 777–791.