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Processing of recombination intermediates by the RecG and RuvAB proteins of *Escherichia coli*

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ABSTRACT

The RuvAB, RuvC and RecG proteins of *Escherichia coli* process intermediates in recombination and DNA repair into mature products. RuvAB and RecG catalyse branch migration of Holliday junctions, while RuvC resolves these structures by nuclease cleavage around the point of strand exchange. The overlap between RuvAB and RecG was investigated using synthetic X- and Y-junctions. RuvAB is a complex of RuvA and RuvB, with RuvA providing the DNA binding subunit and RuvB the ATPase activity that drives branch migration. Both RuvA and RecG form defined complexes with each of the junctions. The gel mobilities of these complexes suggests that the X-junction attracts two tetramers of RuvA, but mainly monomers of RecG. Dissociation of the junction in the presence of ATP requires high levels of RuvAB. RecG is shown to have a much higher specific activity to the extent that very little of this protein would be required to match RuvAB *in vivo*. Both proteins also dissociate a Y-junction, which is consistent with helicase activity. However, RecG shows no ability to unwind more conventional substrates and the suggestion is made that its helicase activity is directed towards specific DNA structures such as junctions.

INTRODUCTION

Recombination catalysed by the RecA protein of *Escherichia coli* proceeds via a number of discrete steps in which homologous DNA molecules first pair and exchange strands to form a symmetrical four-stranded structure often referred to as a Holliday junction (Fig. 1A). Branch migration of the junction extends the region of heteroduplex DNA, while symmetrical cleavage around the point of strand exchange produces mature recombinants of the 'patch' and 'splice' type predicted by genetic crosses. The pairing and strand exchange reactions are catalysed by RecA and are reasonably well understood (1, 2). RecA polymerises on single-stranded DNA in a 5′-3′ direction to form a helical nucleoprotein filament that can extend to duplex regions. The RecA-DNA filament initiates pairing with a second DNA molecule which is drawn into the filament in a search for homologous contacts. Pairing involves the formation of three- or four-stranded DNA and leads directly to strand exchange as hydrogen-bonding is switched between complementary strands (2–5).

The later stages of recombination involve the three *ruv* genes (6–8). The products of *ruvA* and *ruvB* together catalyse branch migration of Holliday junctions. RuvA is a DNA binding protein which forms a specific and highly stable complex with junction DNA. This complex is recognised by RuvB which drives migration of the junction in a reaction that depends on hydrolysis of ATP (9–13). A third gene, *ruvC*, encodes a nuclease that resolves the junction into recombinant products (14–17).

The enzymatic activities of the RecA and RuvABC proteins define a possible pathway of recombination that takes homologous DNA substrates through defined intermediates into mature recombinants (12, 16). However, the situation *in vivo* is complicated by a functional overlap between the Ruv proteins and the product of *recG*. This overlap was revealed by the discovery that recombination is reduced about 500-fold in *ruv recG* strains, but only 2- to 3-fold in the single mutants (18). The *recG* locus forms part of the *spoT* operon and encodes a 76 kDa protein that belongs to a family of RNA and DNA helicases (19, 20). The gene product has been purified and shown to be a strong DNA-dependent ATPase (21). RecG binds specifically to Holliday junctions, and dissociates these junctions by catalysing branch migration, like RuvAB. However, it does not appear to cleave junctions (21).

The genetic evidence indicates that RecG is just as efficient as RuvAB in terms of producing recombinants in genetic crosses. However, the same cannot be said for repair of damaged DNA since *ruv* mutants are far more sensitive to UV light than *recG* mutants (6, 18, 19, 22). In this paper we present further studies on the branch migration activities of these proteins. We show that RecG has a much higher specific activity than RuvAB and present evidence that it functions as a structure-specific DNA helicase.

MATERIAL AND METHODS

Strains and plasmids

E.coli strain GS566 is GTI265 (*ruvAB recA*) transformed with the temperature-sensitive and runaway-replication *ruvA*$^+$ construct, pGTI25 and p*cI857* (11). The *ruvA* gene in pGTI25

is under the control of the λ_{pL} promoter. GS1454 is a *ΔrecG263::kan* derivative of JM101 (23). It was made by transducing JM101 to kanamycin resistance with phage P1 grown on CF3324 (*ΔrecG263::kan*) (24). GS1482 is GS1454 transformed with pGTI19, a derivative of pUC19 carrying *ruvB*$^+$ under control of the vector *lac* promoter (7). GS1269 is strain BL21(DE3) p*lysS* (25) transformed with the *recG*$^+$ plasmid, pGS772 (21).

Media and general procedures

Cultures were grown in LB broth with antibiotic selection for plasmids as described (19). Methods for gel purification of DNA, ^{32}P labelling of 5′ ends with T4 kinase and 3′ ends with Klenow polymerase followed published procedures (26). The isolation of single stranded pGEM-7Zf(+) phagemid DNA (Promega) and polymerase chain reactions (PCR) with *AmpliTaq* polymerase (Perkin Elmer Cetus) followed recipes and protocols provided by the suppliers.

Proteins

RuvA was purified from strain GS566 using the procedures described by Tsaneva et al. (11). The yield was 4.1 mg of protein from 5 litres of induced cells. RuvB was also purified as described before (11) except that the *ΔrecG* strain GS1482 was used to remove any possibility of contamination with RecG. The yield was 3.0 mg protein from 4 litres of induced cells. RuvA and RuvB were estimated to be >97% pure as estimated by densitometry of Coomassie blue stained SDS-PAGE gels. RecG was purified from strain GS1269 by a modification of the procedure described by Lloyd and Sharples (21) which involved substituting a Mono Q column (Pharmacia) for hydroxylapatite in the final fractionation step. Details of this modification will be presented elsewhere (manuscript in preparation). Protein concentrations were determined by the Bradford method using a BioRad protein assay kit and bovine serum albumin as standard.

Synthetic junctions

X-junction, Y-junction and linear duplex DNA substrates (Fig. 1B) were made by annealing the appropriate synthetic oligonucleotides as described (27). The sequences used were 1 (5′-GACGCTGCCGAATTCTGGCTTGCTAGGACATCTT-TGCCCACGTTGACCC-3′), 2 (5′-TGGGTCAACGTGGGCA-AAGATGTCCTAGCAATGTAATCGTCTATGACGTT-3′), 3 (5′-CAACGTCATAGACGATTACATTGCTAGGACAT-GCTGTCTAGAGACTATCGA-3′), 4 (5′-ATCGATAGTCTC-TAGACAGCATGTCCTAGCAAGCCAGAATTCGGCAGC-GT-3′), 5 (5′-GGGTCAACGTGGGCAAAGATGTCCTAG-CAAGCCAGAATTCGGCAGCGTC-3′), and 6 (5′-TGGGT-CAACGTGGGCAAAGATGTCCGGACATGCTGTCTAGA-GACTATCGA-3′). 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 the values are approximate due to the low concentration.

Gel retardation assays

Reaction mixtures (20 μl) contained ^{32}P-labelled junction or linear duplex DNA (~0.5 μM) in binding buffer (50 mM Tris–HCl, pH 8.0, 5 mM EDTA, 1 mM DTT, 100 μg/ml bovine serum albumin). Proteins were added as indicated and after 15 min on ice, 5 μl loading buffer (40 mM Tris–HCl, 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–HCl, pH 8.0, 3.3 mM sodium acetate, 2 mM EDTA). Electrophoresis was at room temperature for 1h 45 min at 160 V with continuous circulation of buffer. Gels were dried on Whatman 3MM paper and autoradiographed.

Dissociation of junction DNA

Reaction mixtures (20 μl) contained junction or linear duplex DNA (~0.3–0.8 μM) in reaction buffer (50 mM Tris–HCl, pH 8.0, 1 mM DTT, 5 mM $MgCl_2$, 100 μg/ml bovine serum albumin, and ATP as specified) and various amounts of protein sample. ATP regeneration, when used, was provided by including 20 mM phosphocreatine and 6 units per ml phosphocreatine kinase in the reaction. Reactions were incubated at 37°C as required 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 10% native polyacrylamide gels at 160 V for 3.5 h, using a Tris-borate buffer system (27). Gels were dried and autoradiographed. X-ray films were scanned by laser densitometry (Molecular Dynamics) to quantify the results.

DNA helicase substrate

A 182 bp DNA fragment spanning the multiple cloning site of the phagemid pGEM-7Zf(+) (Promega) was made using M13 universal forward sequencing primer (17 mer, Pharmacia) and SP6 promoter primer (19 mer, Promega) in a standard polymerase chain reaction. The fragment was denatured and 200 ng annealed to 4 μg single-stranded pGEM-7Zf(+) DNA as described (28). The 3′ end of the annealed fragment was extended by two nucleotides using Klenow polymerase and [α^{32}P] dGTP and [α^{32}P]dCTP (Amersham) and the substrate was passed through a NICK^{TM} spin column (Pharmacia) to remove unincorporated label. Excess circular single-stranded DNA remains in the preparation.

Helicase assays

Reaction mixtures (20 μl) contained substrate DNA (~5 ng of the annealed 182 mer strand) in reaction buffer (20 mM mM Tris–HCl, pH 7.5, 2 mM DTT, 15 mM $MgCl_2$, 100 μg/ml bovine serum albumin), 2 mM ATP (with ATP regeneration), and various amounts of protein sample. Reactions were incubated at 37°C for 30 min before deproteinisation as above. The DNA products were resolved by electrophoresis on 8% native polyacrylamide gels and visualised by autoradiography.

RESULTS

Binding of RuvA and RecG to synthetic X- and Y-junction DNA

The DNA binding specificity of RuvAB is provided by the 24 kDa RuvA subunit (10, 13). We used a simple band-shift assay to compare the binding of RuvA and RecG to synthetic X- and Y-junctions (Fig. 1B). The X-junction has a homologous core of 12 bp which is free to branch migrate and provides therefore a close mimic of a Holliday junction (16). Both proteins have been shown to bind X-junctions (10, 21). The data in Figure 2 (lanes b–d and l–n) show that under identical conditions, two complexes with defined mobilities are formed in each case. These were detected over a similar range of RuvA and RecG concentrations. Previous studies (10) overestimated the amount of RuvA needed to form these complexes by some 8-fold due

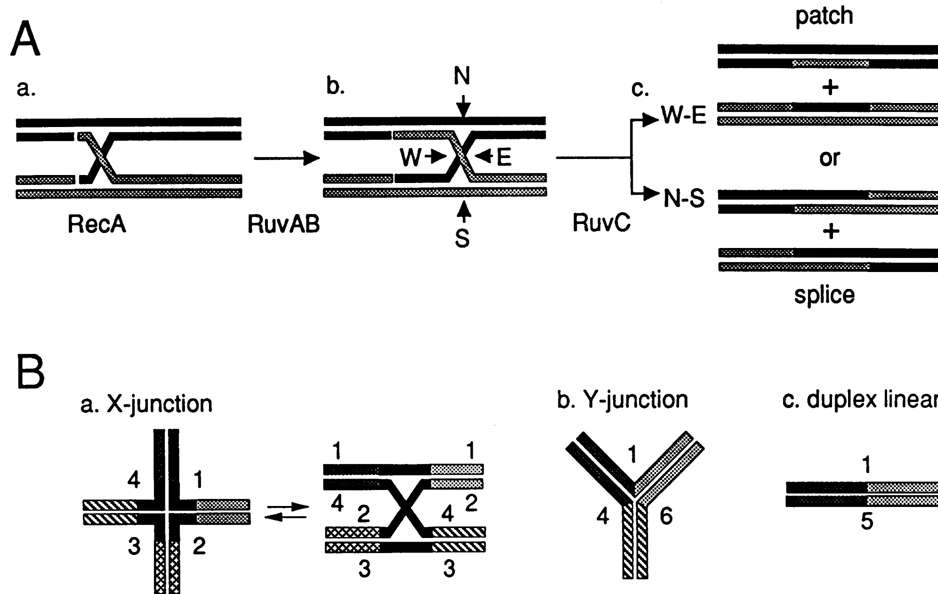

Figure 1. A. Stages of recombination between two duplex DNA molecules showing (a) homologous pairing and strand exchange initiated by RecA (b) branch migration of the Holliday junction by RuvAB and (c) resolution of the symmetrical junction by RuvC in either of the two possible orientations to give recombinants of the patch and splice type. **B**. DNA substrates for recombination assays made by the annealing of 49−51 mer synthetic oligonucleotides. The X-junction is shown also shown in the form of a Holliday intermediate. The sequences of the strands numbered are those identified in Materials and Methods.

to anomalous results with ovalbumin as a standard in the protein assay (11). With RecG, a substantial amount of the junction is incorporated into a defined complex with relatively small amounts of protein. This complex remains the major product as the protein concentration is increased further (Fig. 2A, lanes b−d, and data not shown). A second, complex with reduced mobility was detected with 150 nM protein, but only in trace amounts (lane d). In contrast, the faster migrating complex formed with RuvA is a minor species and all of the junction is shifted to a slower migrating complex as the protein concentration was increased from 7.5 nM to 30 nM (Fig. 2B, lanes l and m). The relative mobilities of the RuvA and RecG complexes is also informative. The minor complex formed with RuvA migrates more slowly than the major complex formed with RecG. Since RuvA (24 kDa) is much smaller than RecG (76 kDa), this observation suggests that it binds as a multimer. Previous studies have shown that RuvA forms a tetramer in solution (11, 29). From the band-shifts observed we suspect that a single tetramer produces the minor complex seen at lower concentrations of protein and that two of these tetramers assemble on the junction to produce the major complex. If our interpretation is correct, then RecG would seem to bind mainly as a monomer.

Both RuvA and RecG also form defined complexes with the Y-junction (Figs. 2 lanes f−h, and p−r). No binding to linear duplex DNA was detected with the highest concentrations of protein used (lanes j and t), from which we deduce that the complexes are the result of a specific interaction with the junction. The complexes formed have almost the same mobility as those formed on the X-junction. Presumably, both types of junction attract similar assemblies of proteins. However, with RuvA the complexes are less stable than those formed with the X-junction and tend to dissociate during the electrophoresis to give a smear of faster migrating radioactivity. By this definition, the RecG complexes are generally less stable, but the same trend is observed.

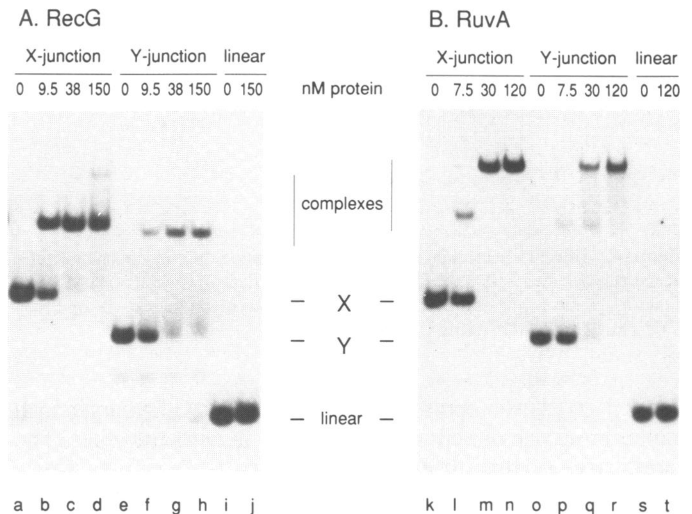

Figure 2. Gel retardation assay showing binding of RecG and RuvA proteins to synthetic X- and Y-junctions. Reaction mixtures containing the purified proteins as indicated were mixed with ^{32}P-labelled substrate DNA (~0.5 μM) on ice for 15 min before separating the complexes on a 4% polyacrylamide gel as described.

Dissociation of X-junctions

RuvAB and RecG are both able to dissociate the synthetic X-junction described above into duplex products with single strand ends (10, 21). These products are the half-junctions expected if the junction were to be forced through the non-homologous arms. Although both proteins require ATP to drive the reaction, we noticed a considerable difference in the concentration required for maximal activity. RecG works best with 5−10 mM ATP (Fig. 3, lanes, i and j) whereas RuvAB is totally inhibited at these concentrations and has an optimum between 0.5 and 2.5 mM

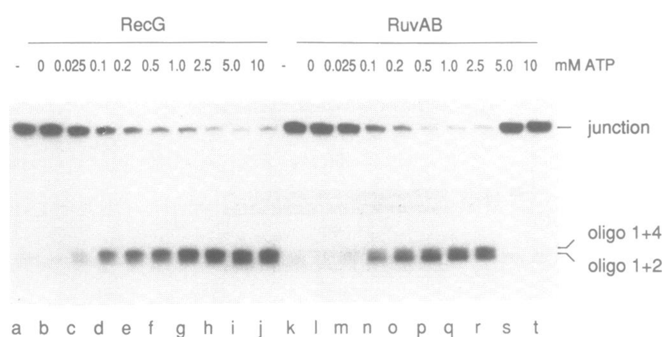

Figure 3. Effect of ATP concentration on dissociation of synthetic X-junction DNA by RecG and RuvAB. Reaction mixes contained ATP at the concentrations indicated, ~0.4 μM X-junction DNA and either no protein (lane a), 500 nM RecG (lanes b–j), 125 nM RuvA (lane k), or 125 nM RuvA plus 500 nM RuvB (lanes l–t). Incubation was for 30 min at 37°C before deproteinising and separating the products on a 10% acrylamide gel.

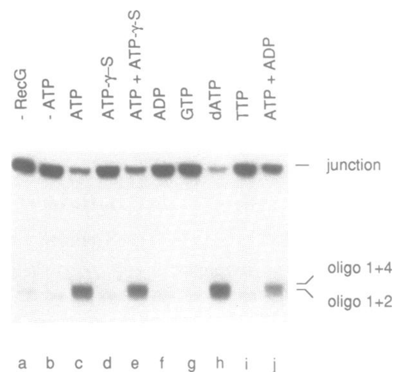

Figure 4. Effect of nucleotide cofactors on dissociation of X-junctions by RecG. Reactions contained ~0.8 μM junction DNA alone (lane a) or with 500 nM RecG (lanes b–j) and 1 mM of the nucleotide cofactor indicated (lanes c–i), or 1 mM ATP plus 2 mM ADP (lane j).

(lanes p–t). At sub-optimal concentrations, RecG favours branch migration in one direction while RuvAB favours the other. The significance of this difference is unclear.

The high level of ATP needed by RecG to drive the reaction to completion is somewhat surprising. Since RecG has a very potent ATPase activity (21), we considered the possibility that the reaction is inhibited by the accumulation of ADP. No products were formed when ATP was replaced by the non-hydrolysable ATP-γ-S (Fig. 4, lane d), or with ADP (lane f). Addition of 2 mM ADP to reactions containing 1 mM ATP resulted in a substantial reduction in activity (lane j). These results demonstrate that ATP hydrolysis is necessary for dissociation of junctions by RecG and suggest that the rapid accummulation of ADP is probably responsible for the reduced activity at ATP concentrations below 5 mM. The data in Figure 4 also show that ATP can be replaced by dATP (lane h), but not by GTP (lane g) or TTP (lane i).

Effect of protein concentration

Dissociation of a synthetic X-junction seems to require very much higher concentrations of RuvAB than RecG (10, 21). However, previous studies were conducted in different laboratories with different preparations of X-junction. We therefore re-examined the protein requirement in greater detail using a single preparation of substrate DNA. We first titrated RuvA against RuvB and found that with 50–500 nM RuvA in the reactions, dissociation activity was related to the concentration of RuvB alone up to 1 μM (data not shown). This stoichiometry for the RuvAB reaction is much the same as reported before (10) except that the latter studies overestimated the amount of RuvA and RuvB used by some 8-fold as already described. For the comparison with RecG, we used a constant level of 200 nM RuvA in the RuvAB reactions and varied the concentration of RuvB. Figure 5A shows the results of an experiment using ATP at 5 mM for RecG and 1 mM for RuvAB. Very similar results were obtained when the ATP concentrations were halved in each case, or when both reactions used ATP at 1mM with ATP regeneration (data not shown). The RuvAB activity reached a maximum with 350 nM RuvB with no further increase up to 1.4 μM (Fig. 5A lane p and data not shown). Below 350 nM the activity declined very sharply and none was detected below 44 nM (lanes j–o). In contrast, the RecG activity reached a plateau with 0.6 nM protein and activity was still detectable at 6 pM (Fig 5A, lanes b–h). From the densitometric tracings (Fig 5B) we calculated that dissociation of 50% of the junction needs about a 1000-fold more RuvB than RecG.

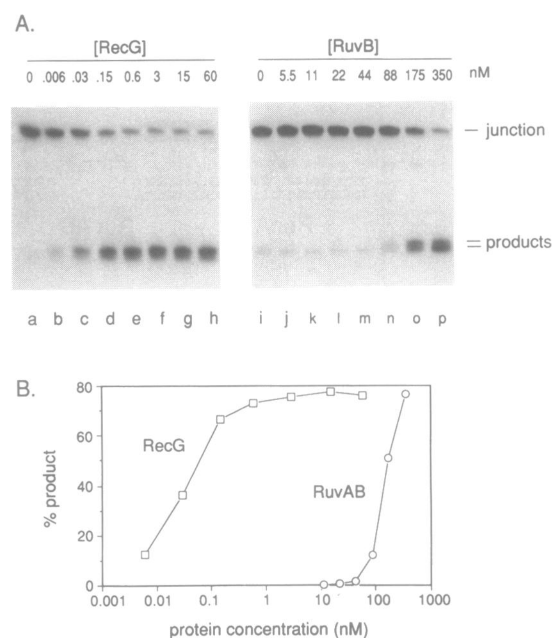

Figure 5. A. Autoradiograph showing the effect of RecG and RuvB concentration on dissociation of X-junctions. Reaction mixtures contained ~0.3 μM X-junction DNA and RecG at the concentrations indicated (lanes b–h), or RuvA at 200 nM and RuvB at the concentrations indicated (lanes i–p). RuvA protein was added to the relevant reactions 5 minutes before addition of RuvB. Incubation was for 30 min at 37°C before deproteinising and separating the products on a 10% acrylamide gel as described. **B.** Quantification of the autoradiographs (A) by laser densotometry. The% product in the control lanes without RecG or RuvB has been subtracted from the values plotted.

Kinetics of the dissociation reaction

To compare the speed of dissociation, we used RecG and RuvB at 500 nM (with 200 nM RuvA also present in the RuvB reaction) and ATP at 1 mM with ATP regeneration. The reactions were

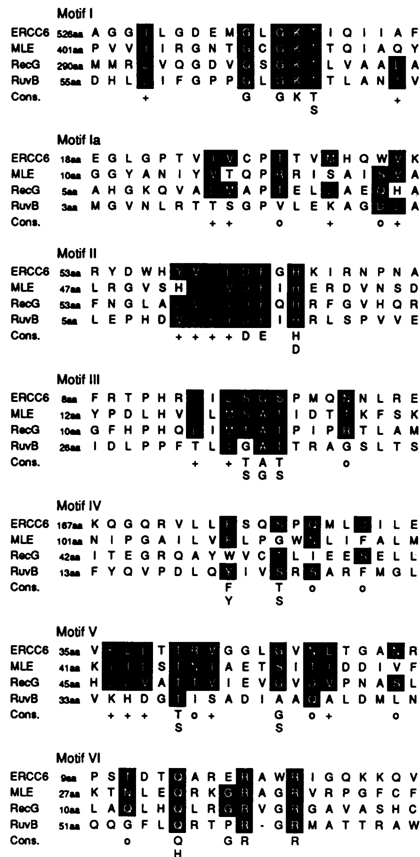

Figure 6. Alignment of the amino acid sequences of RecG and RuvB (20, 37) with the seven conserved DNA/RNA helicase motifs defined by Gorbalenya (34). The sequences of ERCC6 (38) and MLE (39) are included for comparison. All four proteins fall into the DExH sub-family. The amino acids outlined in black match the consensus (Cons.) sequence (34). Hydrophobic residues (+) are I, L, V, M, F, Y, and W. Charged or polar residues (o) are S, T, D, E, N, Q, K. The number of amino acids (aa) between the motifs is indicated at the start of each block. Numbers at the start of motif I refer to the number of amino acids residues from the N-terminus.

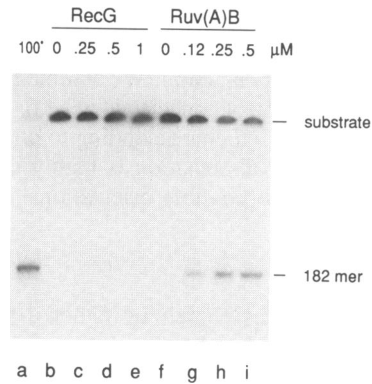

Figure 7. Assay of DNA helicase activity of RecG and RuvAB. Reaction mixtures (20 μl) contained substrate DNA as described in Methods and either RecG at the concentrations indicated (lanes b–e), or 30 nM RuvA plus RuvB at the concentrations indicated (lanes f–i). Incubation was for 30 min at 37°C before deproteinising and separating the products on an 8% polyacrylamide gel. A sample of the substrate was boiled to provide a control (lane a).

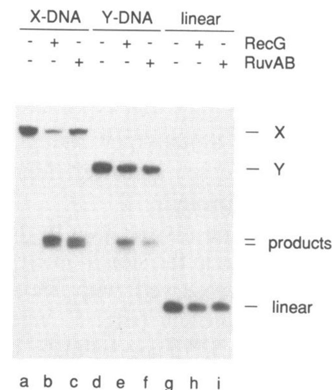

Fig. 8. Autoradiograph showing dissociation of X- and Y-junctions by RecG and RuvAB. Reaction mixtures contained ~0.6 μM substrate DNA and either 500 nM RecG or 500 nM RuvB plus 200 nM RuvA as indicated, with ATP at 5 mM for the RecG reactions and 1mM for the RuvAB reactions. Incubation was for 15 min before deproteinising and separating the products as described.

incubated in parallel and were sampled at intervals to measure the formation of products. The RecG reaction was extremely rapid, with 50% of the junction dissociated within 20 seconds. The activity reached a plateau at 72% dissociation within 2 to 3 minutes. RuvAB worked more slowly. It needed three minutes to dissociate 50% of the junction and 20 minutes to reach the same plateau as RecG (data not shown).

DNA helicase activities

Dissociation of the synthetic X-junction used in these experiments presumably involves a DNA helicase activity to melt the heterologous arms designed into the structure to prevent spontaneous branch migration (Fig. 1B). We compared the sequences of RecG and RuvB and found that both have a number of motifs that are well conserved in the DExH family of DNA and RNA helicases (Fig. 6). Recent studies have shown that RuvAB is indeed able to function as a DNA helicase (30). To test RecG, a 182 nucleotide fragment of DNA complementary to the circular ϕX174 viral strand DNA was used to make a DNA helicase substrate as described in Materials and Methods. RuvAB protein was able to displace the annealed fragment at relatively low concentrations of protein (Fig. 7, lanes g–i). However, no displacement was seen with RecG (lanes c–e). In other reactions we have used a 52 mer oligonucleotide to generate a similar substrate. Again, unwinding was detected with RuvAB but not with RecG (data not shown).

The possibility remains that RecG has a DNA helicase activity that is directed at a specific structure such as a junction in duplex DNA. We tested this possibility using the Y-junction since we already knew that RecG forms specific complexes with this DNA. When the Y-junction was incubated with RecG in the presence of ATP we observed the formation of a DNA band (Fig. 8, lane e) with the same mobility as the upper of the two products of dissociation of the X-junction (lane b). This product corresponds to the partial duplex molecule produced by annealing oligonucleotides 1+4 (Fig. 1B) (21). The same product was observed in the RuvAB reaction, but at a much reduced level (lane f). We examined the effect of protein concentration on this reaction and again found that RecG was much more active than

RuvAB (data not shown). By exposing the X-ray films for longer periods, a trace of a second product with slightly grater mobility could be seen in the RecG reaction. We assume this corresponds to the product of annealing oligonucleotides 1+6. In none of these reactions did we see a band corresponding to oligonucleotide 1, which suggests that the dissociation is highly asymmetric and favours one of the three possible orientations.

DISCUSSION

We used DNA substrates containing either a symmetrical X-junction or a Y-junction to compare the activities of the RecG and RuvAB proteins. Both RecG and RuvA (the latter provides the DNA binding subunit of RuvAB) form defined complexes with each of these junctions. The ability to bind the Y-junction is very informative, especially since the complexes formed have similar mobilities to those formed on the X-junction and must contain therefore similar assemblies of proteins. It shows that DNA sequence homology between arms of the junction, as in the case of true Holliday junctions, is not essential for DNA binding. The most important feature is the branched structure of the DNA since there is no binding to linear duplex DNA at the protein concentrations used. However, symmetry is also important since it is clear from the increased stability of the complexes that the X-junction is favoured. The data also suggest that each X-junction may bind two tetramers of RuvA. RecG appears to bind largely as a monomer, though it may also bind as a dimer since we could see a slower migrating complex at higher concentrations of protein.

A comparison of junction dissociation by RecG and RuvAB revealed a major difference in the stoichiometry of the reactions. Substantial activity was observed with RuvAB only at high concentrations of RuvB protein (Fig. 5, lanes o and p). The measures of junction DNA were approximate, but it is clear from the levels of protein required that both RuvA and RuvB monomers outnumbered junctions by at least one order of magnitude, probably nearer two in the case of RuvB. The data obtained revealed a very sharp transition from maximal dissociation to zero activity over little more than a 4-fold reduction in the level of RuvB. Changing the level of RuvA had no effect on this transition, at least within the range of 50 to 500 nM. The RecG reaction requires much less protein and activity is detectable when junctions outnumber RecG monomers. If this difference reflects the situation *in vivo*, high levels of RuvAB would be required to match the activity of RecG. Presumably, SOS induction helps to achieve these levels (31, 32). There is evidence that the active form of RuvAB is a complex of a RuvA tetramer and a RuvB dimer (33). We cannot rule out the possibility that there are problems with reconstituting active RuvAB from the purified subunits. In other studies to be reported elsewhere we show that RecG can substitute very effectively for the absence of RuvAB to promote wild-type levels of survival after UV-irradiation (T. Mandal, A.A. Mahdi, G.J. Sharples and R.G. Lloyd, manuscript in preparation). This observation suggests that RecG activity is not a limiting factor despite the poor expression of *recG*. It also favours the idea that RecG has high specific activity relative to RuvAB.

The studies described also show that RecG and RuvAB dissociate a Y-junction. The Y-junction is a static structure and although RecG is more efficient than RuvAB, the net effect in each case is to remove oligonucleotide 6. This requires unwinding of the 24 and 25 bp duplex arms produced by annealing of oligonucleotide 6 to oligonucleotides 1 and 4, respectively (see Fig. 1B). A similar unwinding reaction would be required to melt the heterologous arms of the X-junction. RuvAB has been shown to have a DNA helicase activity that displaces short oligonucleotides annealed to circular single-stranded DNA (30). A comparison of the sequences of RecG and RuvB revealed similarities to the DExH family of RNA and DNA helicases (34). The motifs in RuvB are generally less well conserved than those in RecG and apart from those concerned with binding ATP have received little attention. Motifs I, II and VI are highly conserved (Fig. 6). The glycine residue in motif III is a non-conservative substitution, though this is not exceptional (34). RuvB also contains the sequence MATTRA adjacent to motifVI. This sequence matches the consensus motif VI for superfamily I, which includes DNA helicase II (35). The helicase activity of RuvB is cryptic however, and requires RuvA for its activation. In the case of RecG, we were unable to detect any helicase activity using a conventional substrate. We assume its ability to promote unwinding is restricted to DNA molecules with specialised structures such as junctions. In studies to be described alsewhere (manuscript in preparation), we show that the ability of RecG to dissociate junctions is abolished by a mutation converting the consensus alanine in motif III to a valine. Changes in this motif have been shown to eliminate the helicase activity of eIF-4A, an RNA helicase (36).

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