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Formation, Translocation and Resolution of Holliday Junctions during Homologous Genetic Recombination

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Formation, translocation and resolution of Holliday junctions during homologous genetic recombination

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SUMMARY

Over the past three or four years, great strides have been made in our understanding of the proteins involved in recombination and the mechanisms by which recombinant molecules are formed. This review summarizes our current understanding of the process by focusing on recent studies of proteins involved in the later steps of recombination in bacteria. In particular, biochemical investigation of the *in vitro* properties of the *E. coli* RuvA, RuvB and RuvC proteins have provided our first insight into the novel molecular mechanisms by which Holliday junctions are moved along DNA and then resolved by endonucleolytic cleavage.

1. INITIATION OF RECOMBINATION

A simple scheme which attempts to coordinate the properties of the enzymic activities involved in recombination in *E. coli* is shown in figure 1. Recombination in bacteria, and probably also in eukaryotic organisms, requires the formation of single-stranded DNA (West 1993). Single strands are generated by the combined helicase/nuclease activities of RecBCD enzyme (see accompanying article by G. R. Smith), following cleavage at specific sites known as Chi (Smith 1990; Taylor 1988). Mutations in *recB*, or *recC*, reduce the frequency of recombination but do not block it completely, because in the absence of functional RecBCD, other proteins can provide the single-stranded DNA required for initiation. For example, a DNA helicase such as RecQ (Umezu *et al.* 1990) or a single-strand specific exonuclease such as RecJ, or their combined action, would suffice (Lovett & Kolodner 1989). A similar function could be played by DNA helicase II (*uvrD* gene product) or helicase IV (*helD* gene product) (Matson & George 1987; Mendonca *et al.* 1993; Wood & Matson 1987).

2. PAIRING AND STRAND EXCHANGE

The presence of single-stranded DNA triggers recombination reactions catalysed by the RecA protein. This 38 kDa protein is quite remarkable in that it plays a dual role in recombination by providing: (i) the catalytic activity for homologous pairing and strand exchange; and (ii) the structural framework within which recombination reactions take place (figure 2). In the presence of ATP, RecA binds DNA (either single stranded or gapped duplex) to form a right-handed helical nucleoprotein-filament (West *et al.* 1980; Stasiak *et al.* 1981; Flory & Radding 1982; Stasiak & DiCapua 1982). Recent studies suggest that RecF, RecO, RecR and SSB may facilitate the loading of RecA onto the initiating single strand (Madiraju & Clark 1990, 1991; Umezu *et al.* 1993) and may be considered, therefore, as accessory proteins for RecA. Electron microscopic observations of RecA filaments formed on duplex DNA, in the presence of ATP or a non-hydrolysable ATP-analogue (ATPγS), show that the DNA is underwound as the pitch of the helix is extended to approximately 95Å (Stasiak *et al.* 1981). Thus DNA is stretched from a 3.4Å axial rise per base pair (b.p.) in B-form DNA to a 5.1Å rise per b.p. in the RecA–DNA complex. A similar stretching occurs with single-stranded DNA. This extended and regular filament structure, which contains 6.2 RecA monomers per turn of DNA (18.6 b.p.) (DiCapua *et al.* 1982; Yu & Egelman 1990), represents the active form of RecA. A second, non-extended form of the RecA filament has been observed with single-stranded DNA in the absence of a nucleotide cofactor, though this structure is thought to be inactive (Yu & Egelman 1992; Egelman & Stasiak 1993; Ruigrok *et al.* 1993). The crystal structure of the RecA-helical polymer has been solved at a resolution of 2.3Å, and resembles the inactive filament (Story & Steitz 1992; Story *et al.* 1992; Egelman & Stasiak 1993).

The primary function of RecA is to bring two DNA molecules into close proximity within a single filament, so that the DNA sequences are aligned, ready for strand exchange and the formation of heteroduplex DNA (Howard-Flanders *et al.* 1984; Stasiak *et al.* 1984; West 1992). Pairing is thought to occur by means of multiple random contacts which are facilitated, at least *in vitro*, by the formation of large protein–DNA aggregates that serve to concentrate the DNA (Gonda & Radding 1986). It is likely that the two DNA molecules become interwound within the RecA filament in the form of a multi-stranded DNA helix (figure 2) (see also article by C. M. Radding). The

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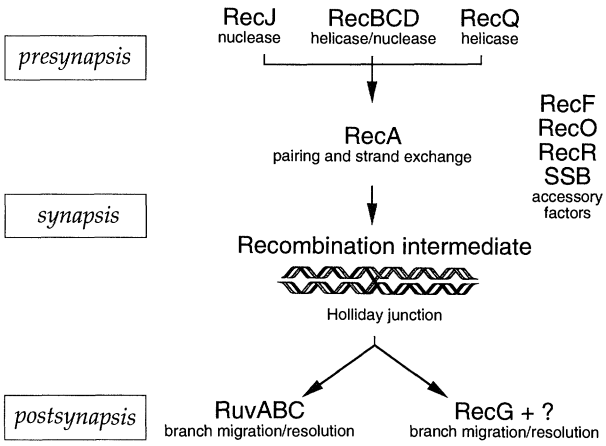

Figure 1. Recombination proteins and their roles in the recombination process. The RecBCD, RecJ and RecQ proteins utilize their nuclease and/or helicase functions to generate regions of single-stranded DNA which are recognized by RecA. The RecF, RecO, RecR and SSB proteins act as accessory proteins which facilitate the interaction between RecA and ssDNA. RecA forms helical nucleoprotein filaments which interact with other duplex DNA molecules leading to homologous pairing and strand exchange. Recombination intermediates, containing Holliday junctions made by RecA are then recognized by RuvAB or RecG, which catalyse branch migration and the formation of heteroduplex DNA. Finally, the Holliday junctions are resolved by RuvC (or an alternative resolvase activity), and the recombinant DNA molecules are repaired by DNA ligase. A number of the indicated proteins, including RecQ, RecA, RuvA and RuvB, are part of the SOS response to DNA damage and induced by UV-irradiation. In addition to their role as general recombination proteins, they are likely to play a particularly important role during recombinational repair.

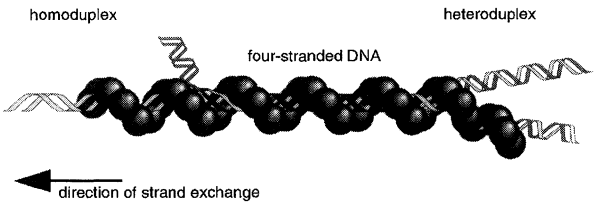

Figure 2. Homologous pairing and strand exchange mediated by RecA protein. The DNA lies within the deep groove of the RecA filament and is stretched (axial rise = 5.1Å compared with 3.4Å in B-form DNA). The two interacting duplexes are proposed to be interwound within the RecA filament. Rotation of the filament along its longitudinal axis causes the naked duplex DNA to be spooled into the filament such that strand exchange occurs (although not shown, this corresponds to a 5′–3′ polarity relative to the single-stranded DNA on which filament formation was initiated). For simplicity, the RecA filament is drawn as only a few protein monomers, in reality it may be much longer. Similarly the diameter of the RecA filament (approximately 100Å) and the length of the DNA are underestimated for diagrammatic purposes. Watson-Crick b.p. and any additional bonds are not shown. Deproteinization of the four-stranded immediate would produce a classical Holliday junction.

reaction then proceeds into its next phase in which strands are exchanged to form heteroduplex DNA. Strand exchange occurs relatively slowly (2–10 b.p. per second), with a defined 5′–3′ polarity (Cox & Lehman 1981; Kahn *et al.* 1981; West *et al.* 1981) and during the course of the reaction large amounts of ATP are hydrolysed by RecA (Kowalczykowski 1991; Roca & Cox 1990).

3. PROCESSING OF RECOMBINATION INTERMEDIATES

Recent work has led to significant advances in our understanding of the late steps of genetic recombination and related recombinational repair processes. It is now known that Holliday junctions made by RecA can be recognized by the RuvA and RuvB proteins which catalyse their movement along DNA (Tsaneva *et al.* 1992*b*). This process, known as branch migration, is required for the formation of heteroduplex DNA. The next step, resolution of the junction to allow the separation of recombinant duplexes that can be repaired by DNA ligase, is catalysed by RuvC protein, a Holliday junction-specific endonuclease (Dunderdale *et al.* 1991; Iwasaki *et al.* 1991; Bennett *et al.* 1993). Surprisingly, mutations in *ruvA*, *ruvB* or *ruvC* do not lead to a recombination defective phenotype (Lloyd 1991), thus providing evidence for the presence of a second pathway for Holliday-junction processing. This pathway is likely to involve RecG, an alternative branch migration protein which is functionally analogous to RuvAB (Lloyd & Sharples 1993*a*, 1993*b*; Whitby *et al.* 1993), and may require a second, as yet unidentified, Holliday-junction resolvase activity (Lloyd 1991).

(*a*) *Branch Migration Catalysed by RuvAB*

Electron microscopic visualization of deproteinized recombination intermediates made by RecA, shows that the two interacting DNA molecules are linked by a crossover or Holliday junction (DasGupta *et al.* 1981; West *et al.* 1983; Müller *et al.* 1992). The structure of this junction appears to be an integral component of subsequent processing reactions. *In vitro*, the RuvA (22 kDa) and RuvB (37 kDa) proteins have been shown to act upon synthetic Holliday junctions, or recombination intermediates made by RecA, and catalyse branch migration (Iwasaki *et al.* 1992; Parsons *et al.* 1992; Tsaneva *et al.* 1992a, 1992*b*; Müller *et al.* 1993*a*; Parsons & West 1993). The RuvB protein is a DNA-dependent ATPase (Iwasaki *et al.* 1989; Parsons & West 1993) which, in the presence of ATP and high concentrations of Mg^{2+} ($\geqslant$ 15 mM) can promote branch migration without the need for RuvA (Tsaneva *et al.* 1992*b*; Müller *et al.* 1993*a*). This result indicates that RuvB is the catalyst of the branch migration reaction, with high levels of Mg^{2+} required to overcome the low binding affinity that RuvB shows for DNA (Müller *et al.* 1993*b*). At lower Mg^{2+} concentrations ($\leqslant$ 10 mM), RuvB binds DNA weakly and branch migration shows an absolute requirement for RuvA (Müller *et al.* 1993*a*, 199*b*). Under these conditions, and in the presence of RuvA, the stoichiometric requirement for RuvB is reduced 50-fold, to about 20 RuvB monomers per junction (Müller *et al.* 1993*a*; Parsons & West 1993).

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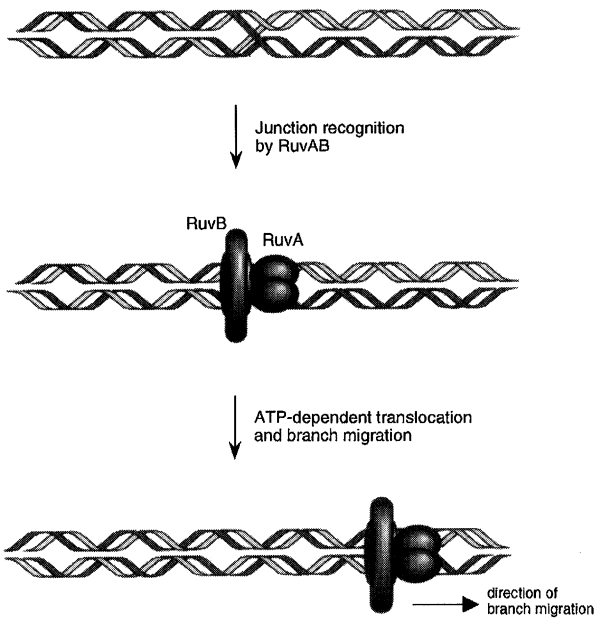

Figure 3. Model for branch migration catalysed by RuvA and RuvB. The RuvA protein specifically targets the RuvB ATPase to the Holliday junction. The RuvA protein is tetrameric while the RuvB protein is likely to take the form of a ring structure which encompasses the DNA. It is not at present known whether one or both DNA helices pass through the hollow cavity in the RuvB ring structure. Translocation of the RuvAB complex, coupled with DNA unwinding, is proposed to lead to the movement (branch migration) of the Holliday junction along DNA.

How does RuvA reduce the requirement for RuvB and thereby facilitate RuvB-mediated branch migration? The answer to this question was provided by band-shift assays which showed that RuvA, which interacts with RuvB in solution (Shiba *et al.* 1993), binds specifically to Holliday junctions (Parsons *et al.* 1992). Moreover, in the presence of a non-hydrolysable ATP analogue (ATPγS) it has been possible to detect stable [RuvAB]–Holliday junction complexes using this assay (Parsons & West 1993). In contrast, without RuvA, RuvB failed to bind the junction. Interestingly, the DNA-dependent ATPase of RuvB, which is normally quite low, is activated by the presence of RuvA (Shiba *et al.* 1991), and junction-specific ATPase activity has been detected (Parsons & West 1993). These results indicate that the primary role of RuvA is to target the RuvB enzyme to the site of the junction where it promotes ATP-dependent branch migration.

Until recently, little was known about the mechanics of RuvAB-mediated branch migration. However, a key observation was made recently when Stasiak *et al.* observed that RuvB protein formed doublet ring-like structures on relaxed circular duplex DNA in the presence of ATPγS and 15 mM Mg^{2+} (Stasiak *et al.* 1994). Scanning transmission electron microscopy and three-dimensional image reconstruction indicates that each ring contains six RuvB monomers. The dodecameric ring structure appears to surround the DNA which passes through a deep hollow core. As the RuvB ring structures were observed in the absence of RuvA, it is not, at present, clear whether this structure represents the active form of the protein involved in branch migration. In fact, recent gel-filtration studies indicate that RuvA and RuvB associate in solution to form a structure made up of a RuvA tetramer with a RuvB hexamer (A. M. Mitchell & S. C. West, unpublished data), and further studies will be required to determine whether the active form of RuvB involves a single or a double ring.

A model for branch migration catalysed by RuvAB is presented in figure 3. It proposes that the high affinity binding shown by RuvA targets RuvB directly to the junction point. Here the RuvB ring structure is assembled, although it is not, at present, known whether the ring encompasses one DNA helix or two within its hollow core. RuvAB have been shown to exhibit DNA-helicase activity (5′–3′ relative to single-stranded DNA) (Tsaneva *et al.* 1983). Based on this fact, it is proposed that the helicase activity plays a direct role in branch migration since RuvB tracks along DNA and promotes strand separation followed by reannealing. It is also possible that ATP hydrolysis is directly coupled to DNA unwinding. Interestingly, recent work indicates that RuvAB can dissociate RecA protein from duplex DNA (Adams *et al.* 1994), possibly as a consequence of translocation. This result is consistent with the proposal that RuvAB act after RecA and it has been suggested that RuvAB may play an important role in recycling RecA protein (Adams *et al.* 1994).

(*b*) *Holliday Junction Resolution by RuvC*

Following branch migration, the production of mature recombination DNA molecules requires the resolution of Holliday junctions – this reaction is carried out by the 19 kDa RuvC protein (Connolly *et al.* 1991; Dunderdale *et al.* 1991; Iwasaki *et al.* 1991; Bennett *et al.* 1993). Band-shift assays show that RuvC forms a specific complex with a Holliday junction, indicating that recognition of the junction is structure-specific (Dunderdale *et al.* 1991; Bennett *et al.* 1993). The binding reaction occurs efficiently in the absence of Mg^{2+} (Bennett *et al.* 1993), a result which is surprising given that divalent cations are known to play an important role in the folding of a Holliday junction (see accompanying article by D. M. J. Lilley) (Duckett *et al.* 1990). The reason for this is that RuvC protein directs the folding of the junction upon binding, so that the initial folding-state of the junction is unimportant (R. J. Bennett & S. C. West, unpublished work).

Whereas junction binding is independent of co-factors, resolution requires the presence of Mg^{2+} (Bennett *et al.* 1993; Dunderdale *et al.* 1994) or Mn^{2+} (Shah *et al.* 1994). The cation is needed for nucleolytic activity rather than DNA folding, because it cannot be replaced by other divalent cations such as Ca^{2+} or Zn^{2+}. Resolution by RuvC occurs via the introduction of nicks into two strands of like-polarity (figure 4). The cuts are placed with perfect symmetry in each duplex, so that the product DNA molecules contain nicks that can be ligated by *E. coli* DNA ligase (Bennett *et al.* 1993; Dunderdale *et al.* 1994). Although binding of a Holliday junction by RuvC is structure-specific, the incision reaction exhibits sequence-specificity (Bennett

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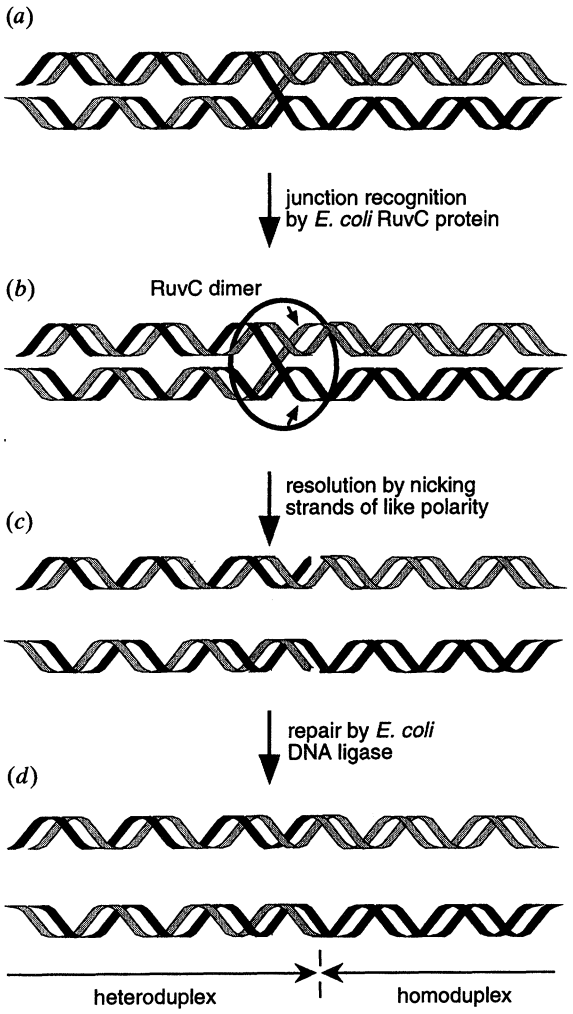

Figure 4. Schematic diagram illustrating the basic features of Holliday junction resolution by RuvC. RuvC protein binds the Holliday junction via structure-specific contacts to form a RuvC-Holliday junction complex (b). Resolution occurs at specific DNA sequences such that nicks are introduced at the same sequence into two strands of like polarity (c). The nicked duplex products are repaired by DNA ligase (d).

et al. 1993; Shah *et al.* 1994). Which may, in part, be relaxed by the presence of Mn^{2+} ions (Shah *et al.* 1994). Although the precise sequences for cleavage are at present unknown, it is likely that branch migration to specific resolution hotspots is required for efficient RuvC-mediated cleavage. This may account for a number of genetic observations which imply a role for RuvAB in Holliday-junction resolution (Mandal *et al.* 1993). Future studies will be required to investigate possible protein–protein interactions between RuvAB and RuvC.

REFERENCES

Adams, D.E., Tsaneva, I.R. & West, S.C. 1994 Dissociation of RecA filaments from duplex DNA by the RuvAB branch migration protein. *Proc. natn. Acad. Sci. U.S.A.* **91**, 9901–9905.

Bennett, R.J., Dunderdale, H.J. & West, S.C. 1993 Resolution of Holliday junctions by RuvC resolvase: Cleavage specificity and DNA distortion. *Cell* **74**, 1021–1031.

Connolly, B., Parsons, C.A., Benson, F.E., Dunderdale, H.J., Sharples, G.J., Lloyd, R.G. & West, S.C. 1991 Resolution of Holliday junctions *in vitro* requires *Escherichia coli ruvC* gene product. *Proc. natn. Acad. Sci. U.S.A.* **88**, 6063–6067.

Cox, M.M. & Lehman, I.R. 1981 Directionality and polarity in RecA protein-promoted branch migration. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6018–6022.

DasGupta. C., Wu, A.M., Kahn, R., Cunningham, R.P. & Radding, C.M. 1981 Concerted strand exchange and formation of Holliday structures by *E. coli* RecA protein. *Cell* **25**, 507–516.

DiCapua, E., Engel, A., Stasiak, A. & Koller, T. 1982 Characterization of complexes between RecA protein and duplex DNA by electron microscopy. *J. molec. Biol.* **157**, 87–103.

Duckett, D.R., Murchie, A.I.H. & Lilley, D.M.J. 1990 The role of metal ions in the conformation of the four-way junction. *EMBO J.* **9**, 583–590.

Dunderdale, H.J., Benson, F.E., Parsons, C.A., Sharples, G.J., Lloyd, R.G. & West, S.C. 1991 Formation and resolution of recombination intermediates by *E. coli* RecA and RuvC proteins. *Nature, Lond.* **354**, 506–510.

Dunderdale, H.J., Sharples, G.J., Lloyd, R.G. & West, S.C. 1994 Cloning, over-expression, purification and characterization of the *E. coli* RuvC Holliday junction resolvase. *J. biol. Chem.* **269**, 5187–5194.

Egelman, E.H. & Stasiak, A. 1993 Electron microscopy of RecA-DNA complexes: Two different states, their functional significance and relation to the solved crystal structure. *Micron.* **24**, 309–324.

Flory, J. & Radding, C.M. 1982 Visualization of RecA protein and its association with DNA: a priming effect of single-strand-binding protein. *Cell* **28**, 747–756.

Gonda, D.K. & Radding, C.M. 1986 The mechanism of the search for homology promoted by RecA protein. Facilitated diffusion within nucleoprotein networks. *J. biol. Chem.* **261**, 13087–13096.

Howard-Flanders, P., West, S.C. & Stasiak, A.J. 1984 Role of RecA spiral filaments in genetic recombination. *Nature, Lond.* **309**, 215–220.

Iwasaki, H., Shiba, T., Makino, K., Nakata, A. & Shinagawa, H. 1989 Overproduction, purification, and ATPase activity of the *Escherichia coli* RuvB protein involved in DNA repair. *J. Bact.* **171**, 5276–5280.

Iwasaki, H., Takahagi, M., Nakata, A. & Shinagawa, H. 1992 *Escherichia coli* RuvA and RuvB proteins specifically interact with Holliday junctions and promote branch migration. *Genes Dev.* **6**, 2214–2220.

Iwasaki, H., Takahagi, M., Shiba, T., Nakata, A. & Shinagawa, H. 1991 *Escherichia coli* RuvC protein is an endonuclease that resolves the Holliday structure. *EMBO J.* **10**, 4381–4389.

Kahn, R., Cunningham, R.P., DasGupta, C. & Radding, C.M. 1981 Polarity of heteroduplex formation promoted by *Escherichia coli* RecA protein. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4786–4790.

Kowalczykowski, S.C. 1991 Biochemistry of genetic recombination: energetics and mechanism of DNA strand exchange. *A. Rev. Biophys. biophys. Chem.* **20**, 539–575.

Lloyd, R.G. 1991 Conjugal recombination in resolvase-deficient *ruvC* mutants of *Escherichia coli K12* depends on *recG*. *J. Bact.* **173**, 5414–5418.

Lloyd, R.G. & Sharples, G.J. 1993*a* Dissociation of synthetic Holliday junctions by *E. coli* RecG protein. *EMBO J.* **2**, 17–22.

Lloyd, R.G. & Sharples, G.J. 1993*b* Processing of recombination intermediates by the RecG and RuvAB proteins of *Escherichia coli*. *Nucl. Acids Res.* **21**, 1719–1725.

Lovett, S.T. & Kolodner, R.D. 1989 Identification and

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

purification of a single-stranded DNA-specific exonuclease encoded by the *recJ* gene of *Escherichia coli*. *Proc. natn. Acad. Sci. U.S.A.* **86**, 2627–2631.

Madiraju, M.V.V.S. & Clark, A.J. 1990 Use of recA803, a partial suppressor of *recF*, to analyze the effects of the mutant SSb (single-stranded DNA-binding) proteins *in vivo* and *in vitro*. *Mol. gen. Genet.* **224**, 129–135.

Madiraju, M.V.V.S. & Clark, A.J. 1991 Effect of RecF protein on reactions catalyzed by RecA protein. *Nucl. Acids Res.* **19**, 6295–6300.

Mandal, T.N., Mahdi, A.A., Sharples, G.J. & Lloyd, R.G. 1993 Resolution of Holliday intermediates in recombination and DNA repair: indirect suppression of *ruvA*, *ruvB* and *ruvC* mutations. *J. Bact.* **175**, 4325–4334.

Matson, S.W. & George, J.W. 1987 DNA helicase II of *E. coli*. Characterisation of the single stranded DNA dependent NTPase and helicase activities. *J. biol. Chem.* **262**, 2066–2076.

Mendonca, V.M., Kaiser-Rogers, K. & Matson, S.W. 1993 Double helicase II (*uvrD*)-helicase IV (*helD*) deletion mutants are defective in the recombination pathways of *Escherichia coli*. *J. Bact.* **175**, 4641–4651.

Müller, B., Burdett, I. & West, S.C. 1992 Unusual stability of recombination intermediates made by *E. coli* RecA protein. *Embo J.* **11**, 2685–2693.

Müller, B., Tsaneva, I.R. & West, S.C. 1993*a* Branch migration of Holliday junctions promoted by the *Escherichia coli* RuvA and RuvB proteins: I. Comparison of the RuvAB- and RuvB-mediated reactions. *J. biol. Chem.* **268**, 17179–17184.

Müller, B., Tsaneva, I.R. & West, S.C. 1993*b* Branch migration of Holliday junctions promoted by the *Escherichia coli* RuvA and RuvB proteins: II. Interaction of RuvB with DNA. *J. biol. Chem.* **268**, 17185–17189.

Parsons, C.A., Tsaneva, I., Lloyd, R.G. & West, S.C. 1992 Interaction of *Escherichia coli* RuvA and RuvB proteins with synthetic Holliday junctions. *Proc. natn. Acad. Sci. U.S.A.* **89**, 5452–5456.

Parsons, C.A. & West, S.C. 1993 Formation of a RuvAB-Holliday junction complex *in vitro*. *J. molec. Biol.* **232**, 397–405.

Roca, A.I. & Cox, M.M. 1990 The RecA protein: Structure and function. *Crit. Revs. Biochem. molec. Biol.* **25**, 415–456.

Ruigrok, R.W.H., Bohrmann, B., Hewat, E., Engel, A., Kellenberger, E. & DiCapua, E. 1993 The inactive form of RecA protein: the compact structure. *EMBO J.* **12**, 9–16.

Shah, R., Bennett, R.J. & West, S.C. 1994 Activation of RuvC Holliday junction resolvase *in vitro*. *Nucl. Acids Res.* **22**, 2490–2497.

Shiba, T., Iwasaki, H., Nakata, A. & Shinagawa, H. 1991 SOS-inducible DNA repair proteins, RuvA and RuvB, of *Escherichia coli*: Functional interactions between RuvA and RuvB for ATP hydrolysis and renaturation of the cruciform structure in supercoiled DNA. *Proc. natn. Acad. Sci. U.S.A.* **88**, 8445–8449.

Shiba, T., Iwasaki, H., Nakata, A. & Shinagawa, H. 1993 *Escherichia coli* RuvA and RuvB proteins involved in recombination repair: physical properties and interactions with DNA. *Molec. gen. Genet.* **237**, 395–399.

Smith, G.R. 1990 RecBCD enzyme. In *Nucleic Acids and Molecular Biology*, vol 4 (ed. F. Eckstein & D. M. Lilley), pp. 78–98. Berlin: Springer-Verlag.

Stasiak, A. & DiCapua, E. 1982 The helicity of DNA in complexes with RecA protein. *Nature, Lond.* **299**, 185–186.

Stasiak, A., DiCapua, E. & Koller, T. 1981 Elongation of duplex DNA of RecA protein. *J molec. Biol.* **151**, 557–564.

Stasiak, A., Stasiak, A.Z. & Koller, T. 1984 Visualization of RecA-DNA complexes involved in consecutive stages of an *in vitro* strand exchange reaction. *Cold Spring Harb. Symp. quant. Biol.* **49**, 561–570.

Stasiak, A., Tsaneva, I.R., West, S.C., Benson, C.J.B., Yu, X. & Egelman, E.H. 1994 The *E. coli* RuvB branch migration protein forms double hexameric rings around DNA. *Proc. natn. Acad. Sci. U.S.A.* **91**, 7618–7622.

Story, R.M. & Steitz, T.A. 1992 Structure of the RecA protein-ADP complex. *Nature, Lond.* **355**, 374–376.

Story, R.M., Weber, I.T. & Steitz, T.A. 1992 The structure of the *Escherichia coli* RecA protein monomer and polymer. *Nature, Lond.* **355**, 318–325.

Taylor, A.F. 1988 RecBCD enzyme of *Escherichia coli*. In *Genetic Recombination* (ed. R. Kucherlapati & G. R. Smith), pp. 231–264. Washington: American Society for Microbiology.

Tsaneva, I.R., Illing, G.T., Lloyd, R.G. & West, S.C. 1992*a* Purification and properties of the RuvA and RuvB proteins of *Escherichia coli*. *Molec. gen. Genet.* **235**, 1–10.

Tsaneva, I.R., Müller, B. & West, S.C. 1992*b* ATP-dependent branch migration of Holliday junctions promoted by the RuvA and RuvB proteins of *E. coli*. *Cell* **69**, 1171–1180.

Tsaneva, I.R., Müller, B. & West, S.C. 1993 The RuvA and RuvB proteins of *Escherichia coli* exhibit DNA helicase activity *in vitro*. *Proc. natn. Acad. Sci. U.S.A.* **90**, 1315–1319.

Umezu, K., Chi, N.W. & Kolodner, R.D. 1993 Biochemical interaction of the *Escherichia coli* RecF, RecO, and RecR proteins with RecA protein and single-stranded DNA binding protein. *Proc. natn. Acad. Sci. U.S.A.* **90**, 3875–3879.

Umezu, K., Nakayama, K. & Nakayama, H. 1990 *Escherichia coli* RecQ Protein is a DNA helicase. *Proc. natn. Acad. Sci. U.S.A.* **87**, 5363–5367.

West, S.C. 1992 Enzymes and molecular mechanisms of homologous recombination. *A. Rev. Biochem.* **61**, 603–640.

West, S.C. 1993 The nucleases of genetic recombination. In *Nucleases* (ed. S. M. Linn, R. S. Lloyd and R. J. Roberts), pp. 145–169. New York: Cold Spring Harbor Laboratory Press.

West, S. C. 1994 The processing of recombination intermediates: Mechanistic insights from studies of bacterial proteins. *Cell* **76**, 9–15.

West, S.C., Cassuto, E. & Howard-Flanders, P. 1981 Heteroduplex formation by RecA protein: Polarity of strand exchange. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6149–6153.

West, S.C., Cassuto, E., Mursalim, J. & Howard-Flanders, P. 1980 Recognition of duplex DNA containing single-stranded regions by RecA protein. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2569–2573.

West, S.C., Countryman, J.K. & Howard-Flanders, P. 1983 Enzymatic formation of biparental figure-8 molecules from plasmid DNA and their resolution in *Escherichia coli*. *Cell* **32**, 817–829.

Whitby, M.C., Ryder, L. & Lloyd, R.G. 1993 Reverse branch migration of Holliday junctions by RecG protein: A new mechanism for resolution of intermediates in recombination and DNA repair. *Cell* **75**, 341–350.

Wood, E.R. & Matson, S.W. 1987 Purification and characterisation of a new DNA-dependent ATPase with helicase activity from *Escherichia coli*. *J. biol. Chem.* **262**, 15269–15276.

Yu, X. & Egelman, E. H. 1990 Image analysis reveals that *Escherichia coli* RecA protein consists of two domains. *Biophys. J.* **57**, 555–566.

Yu, X. & Egelman, E. H. 1992 Structural data suggest that the active and inactive forms of the RecA filament are not simply interconvertible. *J molec. Biol.* **227**, 334–346.

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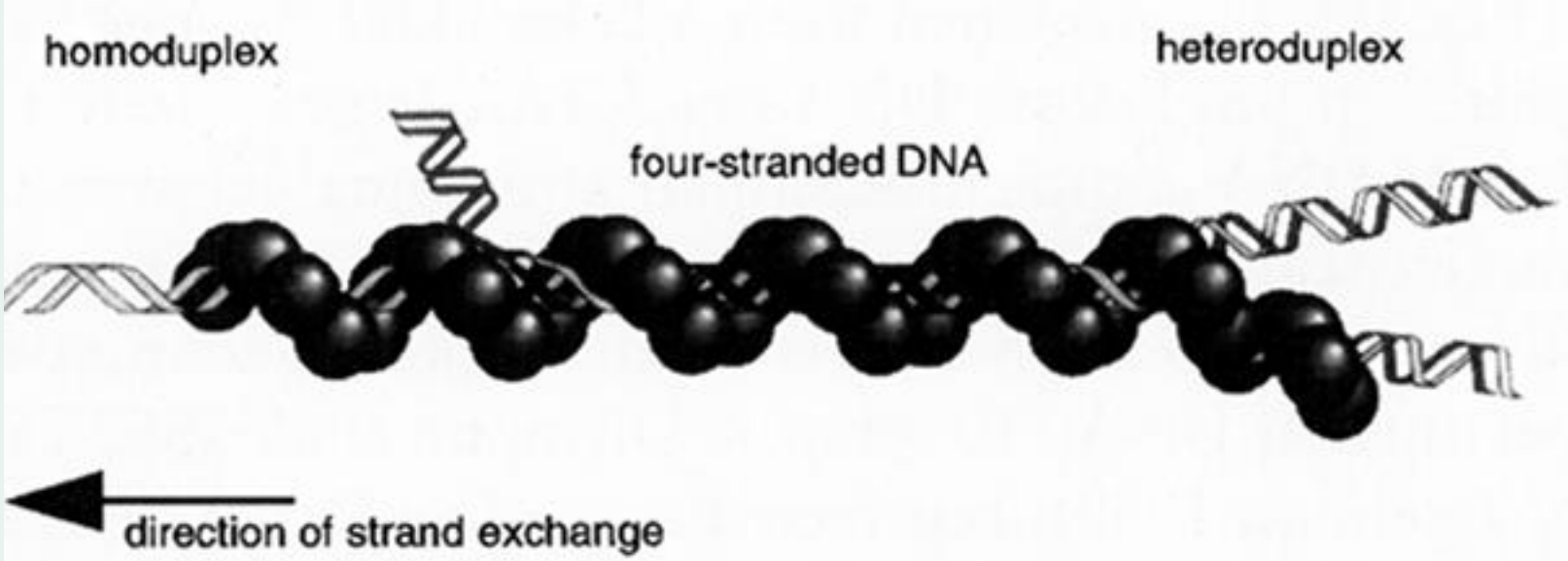

igure 2. Homologous pairing and strand exchange mediated y RecA protein. The DNA lies within the deep groove of the ecA filament and is stretched (axial rise = 5.1Å compared ith 3.4Å in B-form DNA). The two interacting duplexes are roposed to be interwound within the RecA filament. otation of the filament along its longitudinal axis causes the aked duplex DNA to be spooled into the filament such that rand exchange occurs (although not shown, this corre- onds to a 5′–3′ polarity relative to the single-stranded DNA n which filament formation was initiated). For simplicity, ie RecA filament is drawn as only a few protein monomers, reality it may be much longer. Similarly the diameter of ie RecA filament (approximately 100Å) and the length of ie DNA are underestimated for diagrammatic purposes. Vatson-Crick b.p. and any additional bonds are not shown. eproteinization of the four-stranded immediate would roduce a classical Holliday junction.

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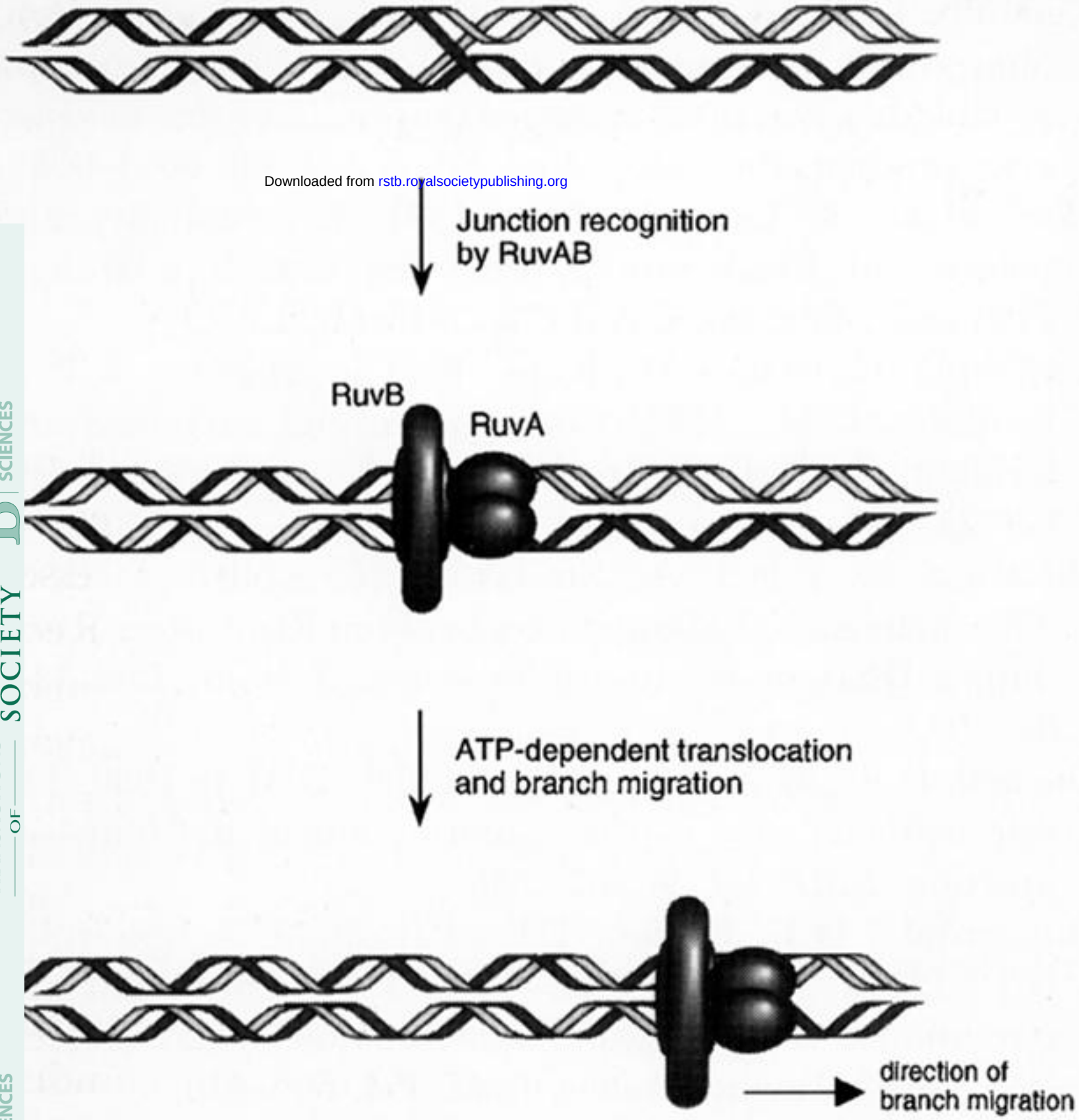

igure 3. Model for branch migration catalysed by RuvA
id RuvB. The RuvA protein specifically targets the RuvB
TPase to the Holliday junction. The RuvA protein is
trameric while the RuvB protein is likely to take the form
a ring structure which encompasses the DNA. It is not at
resent known whether one or both DNA helices pass
irough the hollow cavity in the RuvB ring structure.
ranslocation of the RuvAB complex, coupled with DNA
nwinding, is proposed to lead to the movement (branch
igration) of the Holliday junction along DNA.