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Interplay between DNA replication, recombination and repair based on the structure of RecG helicase

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Recent studies in *Escherichia coli* indicate that the interconversion of DNA replication fork and Holliday junction structures underpins chromosome duplication and helps secure faithful transmission of the genome from one generation to the next. It facilitates interplay between DNA replication, recombination and repair, and provides means to rescue replication forks stalled by lesions in or on the template DNA. Insight into how this interconversion may be catalysed has emerged from genetic, biochemical and structural studies of RecG protein, a member of superfamily 2 of DNA and RNA helicases. We describe how a single molecule of RecG might target a branched DNA structure and translocate a single duplex arm to drive branch migration of a Holliday junction, interconvert replication fork and Holliday junction structures and displace the invading strand from a D loop formed during recombination at a DNA end. We present genetic evidence suggesting how the latter activity may provide an efficient pathway for the repair of DNA double-strand breaks that avoids crossing over, thus facilitating chromosome segregation at cell division.

Keywords: DNA replication; Holliday junctions; helicases; RuvABC; RecBCD; RecG

1. INTRODUCTION

Although we strive to protect our environment and prolong life, we cannot avoid damage to the genetic blueprint on which life depends. Damage is inevitable, extensive and chronic (Lindahl 1996). Organisms survive and reproduce because they have detoxification and repair processes to limit corruption of their DNA and use surveillance mechanisms to make sure cells divide only after they have successfully duplicated their genomes (Jeggo *et al.* 1998; Lindahl & Wood 1999; Tercero & Diffley 2001; Rouse & Jackson 2002). Failure of these systems in humans can be fatal or have catastrophic consequences, as in familial cancers (Venkitaraman 2002) and other heritable or sporadic disorders showing genomic instability or radiation sensitivity (Carney *et al.* 1998; Chakraverty & Hickson 1999; Flores-Rozas & Kolodner 2000; O'Driscoll *et al.* 2003). However some lesions inevitably escape the net and may trigger genetic changes, which underlines the fact that evolution is concerned with survival rather than with exact transmission of the genome.

The dangers posed by unrepaired lesions lie in their ability to block advance of RNA and DNA polymerases, inhibiting transcription of damaged genes and preventing duplication of damaged chromosomes. Stalled transcription complexes present major obstacles to replication and also shield the underlying lesions. Recent studies have highlighted the importance of repair systems that specifically target such complexes (Park *et al.* 2002; Van den Boom *et al.* 2002). However, the mechanisms by which a cell deals with stalled replication complexes are much less clear. Our lack of understanding is compounded by ignorance of what happens to a fork when the replisome meets an obstacle. Lesions directly blocking advance of the replicative helicase are likely to pose very different problems from those preventing the polymerases from synthesizing new DNA strands. Furthermore, owing to the continuous versus discontinuous nature of leading and lagging strand synthesis, lesions preventing synthesis will have different consequences depending on which of the two polymerases is affected. The fate of the replisome complex is also a mystery, despite our understanding of its composition (figure 1*a*). Dissociation of some or all subunits is likely when the fork cannot proceed. However, recent studies in *Escherichia coli* have revealed that a damaged replication fork may be converted to a four-way branched molecule identical at the branch point to the Holliday junction intermediate formed during homologous recombination (figure 1*b*). Furthermore, they have suggested how subsequent processing of this junction by nucleases, helicases and other recombination enzymes might facilitate repair or bypass of the initial blocking lesion and restore a fully fledged fork to restart replication (Cox *et al.* 2000; Marians 2000; Michel 2000; Rothstein *et al.* 2000; McGlynn & Lloyd 2002*b*).

2. RESCUE OF STALLED REPLICATION FORKS IN *ESCHERICHIA COLI*

The rescue of replication forks stalled by unrepaired lesions in the template DNA involves two essential events.

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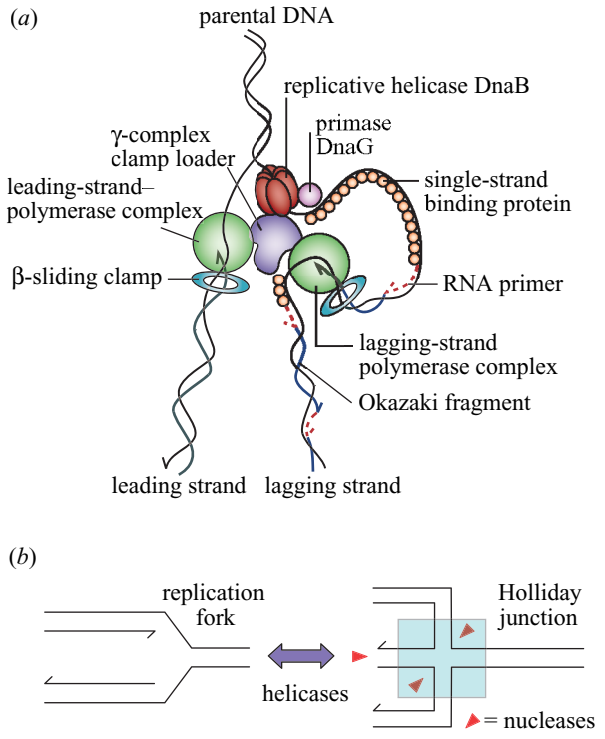

Figure 1. (*a*) Model of the *Escherichia coli* replisome and synthesis of the leading and lagging strands. (Reprinted by permission from *Nature Reviews Molecular Cell Biology* (www.nature.com/reviews) (McGlynn & Lloyd 2002*b*) copyright (2002) Macmillan Magazines Ltd.). Parental DNA strands are unwound by the DnaB replicative helicase. New strands are synthesized by the two DNA polymerase III complexes, one for continuous extension of the leading strand and one for discontinuous synthesis of the lagging strand in the form of Okazaki fragments of 1000–2000 nucleotides, each primed by an RNA primer synthesized by DnaG primase. (*b*) Interconversion of replication fork and Holliday junction structures and identification of potential targets for nuclease attack on the junction.

First, the block to progression must be removed or bypassed. Secondly, replication has to be restarted. Studies by Bénédicte Michel showed that blocking advance of the *E. coli* DnaB replicative helicase can cause chromosome breakage and that this breakage is mediated by the RuvABC Holliday junction resolvase (Seigneur *et al.* 1998; Flores *et al.* 2001). This has led to models of replication restart based on formation of a Holliday junction from a stalled fork (figure 2) and subsequent processing of this structure (Seigneur *et al.* 1998; McGlynn & Lloyd 2002*b*). All rely on the activity of PriA protein, a primosome assembly factor and DNA helicase that can load the DnaB helicase at branched DNA structures, thereby allowing assembly of the entire replisome at damaged forks removed from the normal origin of replication (McGlynn *et al.* 1997; Jones & Nakai 1999; Liu & Marians 1999; Liu *et al.* 1999; Marians 2000; Sandler 2000; Sandler & Marians 2000; Xu & Marians 2003).

A Holliday junction can be formed from a replication fork simply by reversing the direction of fork movement, unwinding the sister duplexes and annealing the nascent strands (figure 1*b*). In the case of a stalled fork, this has the additional effect of exposing the offending lesion, which may allow its repair (figure 2). Seigneur *et al.* (1998) proposed that the nascent duplex DNA spooled out as the fork moves backwards is simply digested by RecBCD nuclease to re-establish a fork structure (figure 2*a*). PriA then loads DnaB, allowing binding of DnaG primase and assembly of DNA polymerase III holoenzyme. Provided the block to replication has been removed, leading and lagging strand synthesis may then restart. Alternatively, the Holliday junction could be targeted and cleaved by the RuvABC resolvase (West 1996), thus breaking or collapsing the fork (figure 2*b*) (Seigneur *et al.* 1998). In this case, RecBCD and RecA recombinase activities (Kowalczykowski 2000) could process the broken arm of the fork to initiate recombination with the intact sister duplex or with a homologue (Horiuchi & Fujimura 1995; Kuzminov 1995; Kogoma 1996). RecA-mediated strand exchange creates a D loop that PriA could exploit to assemble the replicative machinery (Liu & Marians 1999). Resolution of the Holliday junction formed by recombination then re-establishes a fully fledged fork (Seigneur *et al.* 1998). A significant feature of this pathway is that Holliday junction resolution acts both to initiate and complete the recombination reaction. However, to account for the low level of DNA breakage seen in wild-type strains Seigneur *et al.* (1998) proposed that RecBCD normally acts before RuvABC and either digests the DNA or initiates recombination, thus limiting chromosome breakage.

Compelling evidence for an alternative pathway has emerged from the finding that RecG helicase unwinds replication fork structures, catalyses their conversion to Holliday junctions and promotes survival of UV-irradiated cells independently of the RecBCD and RuvABC proteins (McGlynn & Lloyd 2000, 2001*a*,*b*, 2002*a*; McGlynn *et al.* 2001; Bolt & Lloyd 2002; Gregg *et al.* 2002; Jaktaji & Lloyd 2003). RecG was first identified as a protein involved in DNA recombination and repair, a role supported by the subsequent discovery it could catalyse branch migration of Holliday junctions (Lloyd & Sharples 1993). That RecG could also form a Holliday junction from a fork raised the possibility it could act with PriA to promote direct rescue of stalled forks via interconversion of replication fork and Holliday junction structures (figure 2*c*; McGlynn & Lloyd 2000, 2002*a*).

Analysis of the DNA structures targeted by RecG and PriA and dissection of genetic interactions between these proteins indicates that RecG may play a crucial role in the rescue of a stalled fork that has no leading strand at the branch point. Such a structure may arise when a fork runs through a lesion blocking synthesis by the leading strand polymerase (figure 3*a*; McGlynn & Lloyd 2000; Gregg *et al.* 2002; Jaktaji & Lloyd 2003). PriA helicase can target such a fork and by loading DnaB might form an abortive replisome as there would be no 3′OH at the branch point to prime leading strand synthesis. However, RecG has a high affinity for this type of fork (McGlynn & Lloyd 2001*b*) and by converting it to a Holliday junction may facilitate extension of the leading strand via polymerase-mediated template switching and lesion bypass (figure 3*b*) or digestion of the lagging strand extension via a 5′–3′ exonuclease (figure 3*c*). Either mechanism would correct the fork, enabling PriA to assemble a productive replisome. However, once a junction has formed, restart could be redirected in a wild-type strain to RecBCD- and RuvABC-dependent pathways (figure 3*d*). Indeed, such

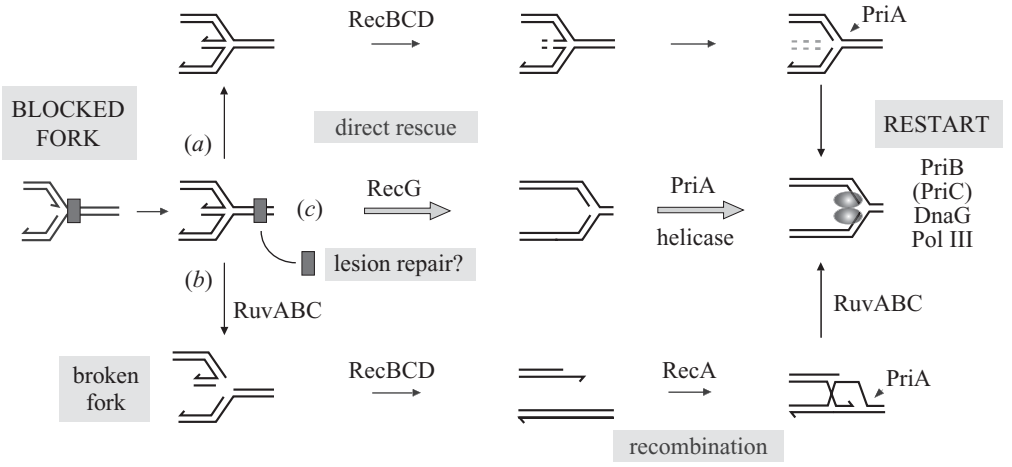

Figure 2. Rescue of replication fork stalled at a lesion in or on the template DNA. Two models are shown, both initiated by reversal or regression of a blocked fork to form a Holliday junction. One (direct rescue) is independent of recombination and requires either (*a*) RecBCD nuclease or (*b*) RecG helicase to facilitate correction of the fork structure and, in addition, both the primosome assembly function and helicase activity of PriA to load DnaB at the corrected fork structure. The second model (recombination) is dependent on RuvABC resolvase to initiate recombination by RecBCD and RecA recombinases, but requires only the primosome assembly activity of PriA to load DnaB at a D loop. A variation of this model would allow RecBCD to initiate recombination from the DNA end spooled from the Holliday junction before RuvABC resolved the structure.

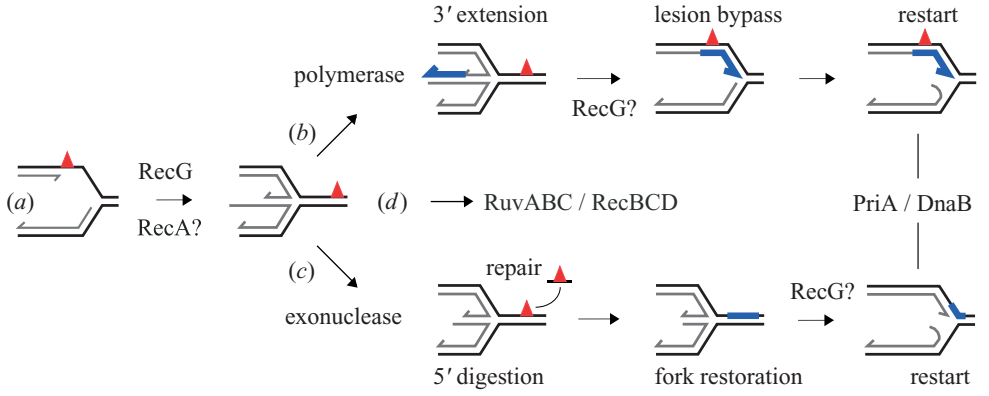

Figure 3. Direct rescue of a replication fork stalled on the leading strand via DNA helicase-mediated interconversion of fork and Holliday junction structures. (*a*) RecG helicase unwinds the extended leading strand and converts the fork to a Holliday junction. (*b*) A DNA polymerase extends the 3′ strand spooled out from the junction using the 5′ end of the lagging strand as a template. Branch migration of the junction mediated by RecG, or possibly RuvAB, bypasses the lesion and resets a fork structure, enabling PriA to load DnaB, thus facilitating replication restart. (*c*) Alternatively, the 5′ extension of the lagging strand spooled from the junction could be digested by a 5′–3′ exonuclease such as RecJ. Provided the lesion was first removed, restart could occur by rewinding the junction back to a fork. (*d*) Resolution of junction by RuvABC breaks the fork, directing restart via RecBCD-dependent recombination (figure 2*b*).

interplay may be necessary to cope with the variety of challenges to replication fork progression (McGlynn & Lloyd 2002*b*).

3. HOLLIDAY JUNCTION FORMATION

The conversion of a replication fork to a Holliday junction requires simultaneous unwinding of both leading and lagging strands, subsequent annealing of these strands and re-annealing of the parental strands. Insight into how these reactions could be catalysed by RecG once the fork DNA is exposed has come from the structure of the *Thermatoga maritima* protein in a complex with a partial fork substrate (figure 4*a*; Singleton *et al.* 2001). A junction can be modelled on RecG such that the point of strand exchange fits around the wedge (figure 4*b*), with two duplex arms held in positions identical to those of the partial replication fork in the RecG-DNA co-crystal (figure 4*a*). Figure 4*c* shows how RecG might also bind to a D loop by targeting the branch point at the 5′ end of the invading strand. We modelled the structure of *E. coli* RecG on the coordinates of the *T. maritima* protein using Swiss-Pdb Viewer (Guex & Peitsch 1997). Apart from the missing N-terminal sequences forming a separate fold in the *T. maritima* protein the *E. coli* RecG structure is essentially identical (figure 4*d*).

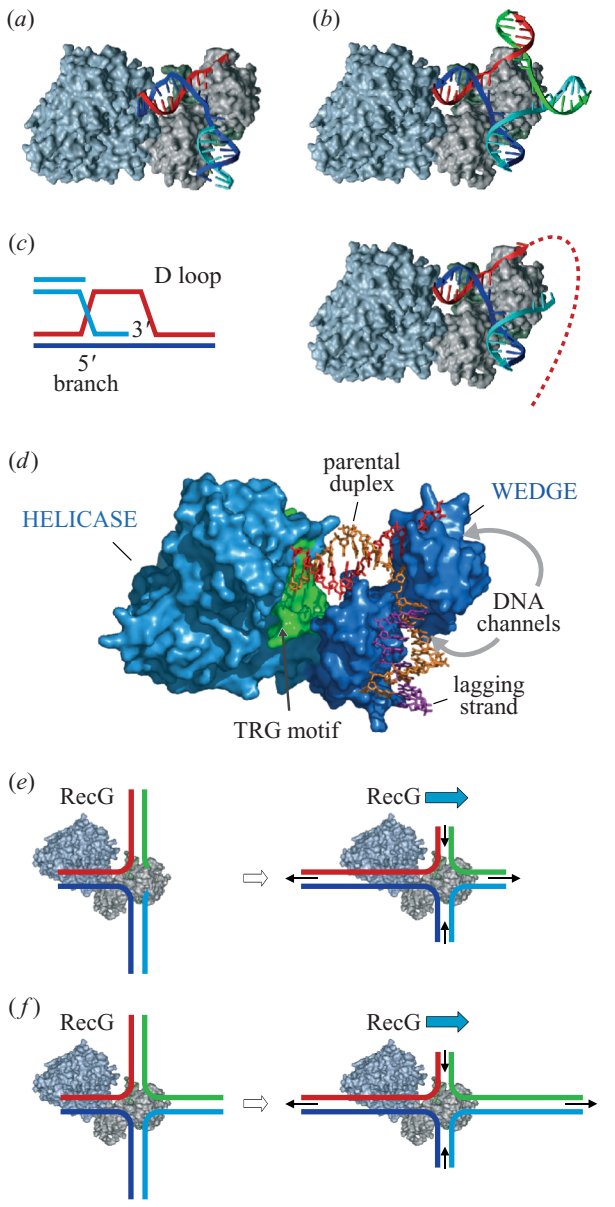

Figure 4. (*a*) Structure of the *Thermatoga maritima* RecG protein in a complex with a partial replication fork structure lacking a leading strand (Singleton *et al.* 2001). (*b*) Superimposition of an open Holliday junction structure as bound by RuvA protein (Hargreaves *et al.* 1998) on the RecG structure. Note that the point of strand separation fits around the wedge domain (panel *d*) and two duplex arms make contact with RecG almost exactly like the fork structure. (*c*) Model of how RecG might target the 5′ branch point of a D loop. Note that the invading strand (coloured cyan) is equivalent to the lagging strand of a fork. The displaced strand of the D loop (coloured red) beyond RecG is shown as a dotted line. (*d*) Structure of *E. coli* RecG modelled on that from *T. maritima*. (*e*) and (*f*) Models for translocation of branched DNA by RecG showing conversion of a replication fork to a Holliday junction (*e*) and branch migration of a Holliday junction (*f*). The black arrows indicate the direction of duplex DNA movement.

The structure of RecG shows it has conserved helicase domains linked to a novel 'wedge' domain providing specificity for binding a branched DNA structure. It has been proposed that the helicase motor acts as a dsDNA translocase, pulling the parental strands of a replication fork through separate channels flanking the wedge, neither wide enough to accommodate duplex DNA (figure 4*d*; Singleton *et al.* 2001). This has the effect of stripping off the nascent strands and allowing the parental strands to re-anneal as suggested by biochemical studies (McGlynn & Lloyd 2000, 2001*b*). The unwound strands may then also anneal, so that as the protein continues to translocate along the rewound parental duplex a Holliday junction forms around the wedge and a 'nascent strand duplex' is spooled out in front (figure 4*e*). This final stage is almost certainly equivalent to the Holliday junction branch migration reaction catalysed by RecG (figure 4*f*; Lloyd & Sharples 1993).

4. dsDNA TRANSLOCATION

RecG sequence alignments and site-directed mutagenesis of conserved residues in the *E. coli* protein, coupled with structural analyses, enabled us to identify a motif in RecG important for helicase activity (Mahdi *et al.* 2003). This motif, named TRG for translocation by RecG, spans residues 606–642 and forms a helical hairpin linked to a loop projecting into the proposed dsDNA binding channel between the helicase and wedge domains (figures 4*d* and 5*a–c*). The helical hairpin places two arginines (R609 and R630, figure 5*b*) in opposing positions where they are stabilized in the ADP-bound crystal structure (Singleton *et al.* 2001) by a network of hydrogen bonds involving a conserved glutamate (E571) from helicase motif VI (figure 5*d*). We believe disruption of this network, triggered by ATP binding or hydrolysis, moves the adjacent loop in the dsDNA binding channel (figure 5*c*) and that a swinging arm motion of this loop drives DNA translocation, possibly via a ratchet mechanism involving backbone contacts, with the loop alternating between 'up' and 'down' conformations between each translocation step (figure 5*d*).

The path of dsDNA across the protein is not known, but our model suggests contacts are made with the conserved loop (residues 630–642), especially with Q640 (figure 5*b–d*). In plasmid constructs, substitution of this glutamine with alanine, arginine or glutamate eliminates the ability of RecG to promote DNA repair (Mahdi *et al.* 2003). We transferred the allele encoding RecG Q640R to the chromosomal *recG* locus (table 1). It confers sensitivity to mitomycin C (data not shown) and also to UV light, especially in a *ruv* mutant background lacking the RuvABC Holliday junction resolvase (figure 6*a*). This effect is corrected by introducing a plasmid encoding wild-type RecG. A plasmid specifying the Q640R derivative has little effect on survival (figure 6*a*). However, it does affect replication of the plasmid, eliminating the ability of RecG to reduce the copy number (figure 6*b*), as has been seen with mutations that inactivate RecG (Mahdi *et al.* 2003). Together, these data show that *recGQ640R* behaves much like a null allele (Bolt & Lloyd 2002). This is consistent with our finding that the Q640R protein has a much reduced ability to drive branch migration *in vitro*. The rate of unwinding of Holliday junction and fork structures is less than 5% of the wild-type activity (figure 6*c*).

The helicase and wedge domains of RecG form independent folding modules, linked by a single long alpha-

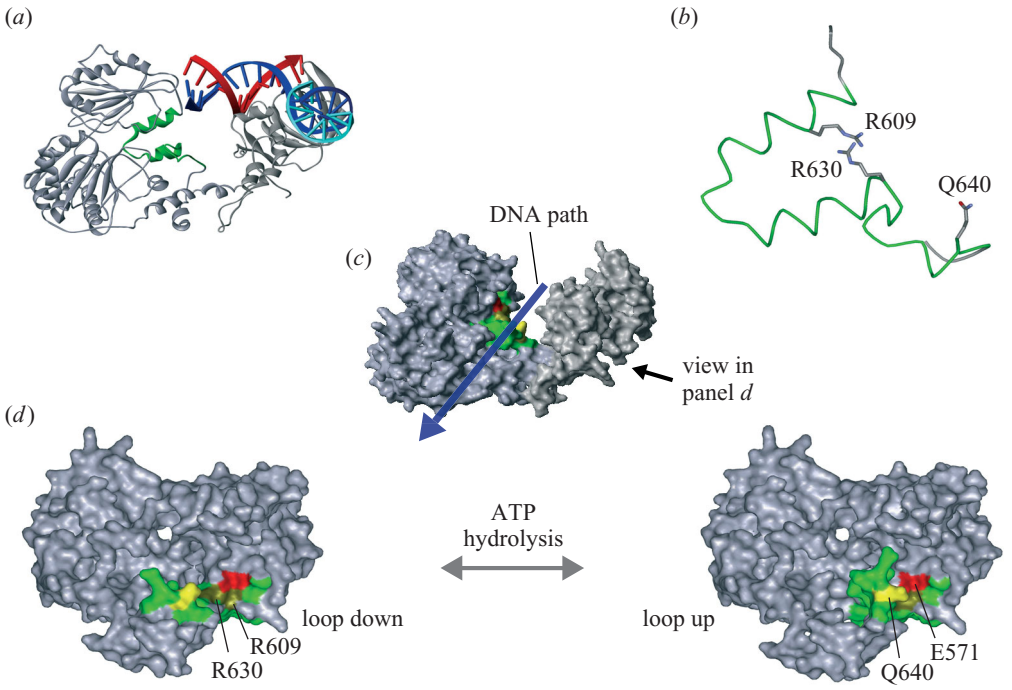

Figure 5. Structure of the TRG motif in RecG. (*a*) Ribbon structure of the modelled *Escherichia coli* RecG in a complex with partial fork DNA, viewed from the end of the lagging strand arm of the fork. The helical hairpin and loop structures of TRG are shown in green. (*b*) Detailed structure of TRG highlighting the two opposed arginines (R609 and R630) and the conserved glutamine (Q640) thought to make contact with the parental DNA duplex. (c) Model of *E. coli* RecG showing projection of the TRG loop (shown in green apart from Q640 which is in yellow and R609 and R630, which are in olive) into the proposed path of duplex DNA translocation. (*d*) Cross-section of *E. coli* RecG viewed from the angle indicated in panel (*c*) showing two possible conformations of the TRG loop based on rotation of the loop around R630. Also highlighted is E571 (red), which hydrogen bonds with R609 and R630.

helix (P205-R245). To gain further insight into the mechanism of translocation we removed the wedge from the *E. coli* protein by deleting residues 49–145, inserting a serine to bridge the gap. Despite the absence of the wedge to direct DNA binding, this construct retains ATPase activity and substantial, though much reduced, ability to dissociate Holliday junctions (data not shown). The protein may simply bind a duplex arm and translocate along the DNA, pushing the junction ahead as it moves rather than actively catalysing strand separation, as has been demonstrated with DnaB helicase (Kaplan & O'Donnell 2002). Although the properties of this RecG deletion support our model the fact that branch migration is reduced indicates that contacts at the DNA branch point facilitate translocation. Such contacts may be mediated via the G16-V17-G18 motif, which in the RecG-fork structure appears to interact with the lagging-strand backbone, aromatic residues F96, F97 and F99, which may stack with bases in the parental duplex, and F75 and Y124, which sit near where the leading strand duplex splits and might stabilize either strand once separated (G. Briggs, unpublished data).

The TRG motif is highly conserved in Mfd protein, which also translocates on dsDNA, but to revive or dislodge stalled RNA polymerase rather than to unwind strands (Park *et al.* 2002). Although there is no structure yet for Mfd, sequence analysis suggests a similar helicase domain to RecG linked via a long alpha-helix to an RNA polymerase interaction domain instead of a strand separation module. Furthermore, substitutions of the arginines equivalent to R609 and R630 in RecG, or of the glutamine equivalent to Q640, prevent Mfd from dissociating stalled RNAP complexes (A. Chambers and N. Savery, personal communication), indicating that it has a similar motor mechanism driving translocation.

5. DNA BRANCH MIGRATION

As RecG translocates a duplex arm of a branched DNA molecule away from the branch point while keeping the wedge firmly placed at the point of strand separation, it is easy to see how it might drive branch migration of at least three different substrates *in vivo*, namely replication forks, Holliday junctions and D loops (figure 4*a*–*c*). We have already described how translocating the parental duplex of a replication fork might drive the fork backwards, converting it to a Holliday junction (figure 4*e*). Similarly, translocating one arm of the four-way symmetrical Holliday junction would drive the branch point in one direction while translocating an adjacent arm would move it in the opposite direction (figure 4*f*). If the Holliday junction derived from a replication fork then translocating either of the two sister duplexes would re-establish a fork structure.

A D loop has two branch points, one at the 5′ end of the invading strand and one at the 3′ end (figure 4*c*). Does RecG target these structures *in vivo*? It certainly unwinds an R loop and reduces the copy number of plasmids that use R loops to initiate replication (figure 6*b*; Vincent *et al.* 1996; Fukuoh *et al.* 1997; Mahdi *et al.* 2003). RecG also limits error-prone replication events thought to be primed by D loop formation (Harris *et al.* 1996). The duplex

Table 1. *Escherichia coli* K-12 strains and plasmids used.

strain/plasmids	relevant properties	source or reference
strains[a]		
MG1665	F$^-$ *rec*$^+$ *ruv*$^+$ *priA*$^+$	Bachmann (1996)
N4239[d]	*ruvA60*::Tn*10*	Jaktaji & Lloyd (2003)
N4256	Δ*recG263*::*kan*	Jaktaji & Lloyd (2003)
N4259	Δ*recG263*::*kan ruvA60*::Tn*10*	P1.*ruvA60*::Tn*10* × N4256 to Tc^r
N4278	*recB268*::Tn*10*	McGlynn & Lloyd (2000)
N4279	*recA269*::Tn*10*	McGlynn & Lloyd (2000)
N4583	Δ*ruvABC*::*cat*	P1.Δ*ruvABC*::*cat* × MG1655 to Cm^r
N4702	Δ*recG263*::*kan recB268*::Tn*10*	P1.*recB268*::Tn*10* × N4256 to Tc^r
N4851	Δ*ruvABC*::*cat recB268*::Tn*10*	P1.*recB268*::Tn*10* × N4583 to Tc^r
N4971	Δ*recG263*::*kan* Δ*ruvABC*::*cat*	P1.Δ*ruvABC*::cat × N4256 to Cm^r
N5059	Δ*recG263*::kan recA269::Tn*10*	P1.*recA269*::Tn*10* × N4256 to Tc^r
N5091	Δ*ruvABC*::*cat recA269*::Tn*10*	P1.*recA269*::Tn*10* × N4583 to Tc^r
N5466	Δ*ruvC*::*cat*	P1.Δ*ruvC*::*cat* × MG1655 to Cm^r
N5469	Δ*recG263*::*kan* Δ*ruvC*::*cat*	P1.Δ*ruvC*::*cat* × N4256 to Cm^r
AM1417	Δ*pyrE*::*dhfr*	this work[b]
AM1418	Δ*recG263*::*kan* Δ*pyrE*::*dhfr*	P1.Δ*recG263*::*kan* × AM1417 to Km^r
AM1432	$recG_{Q640R}$ pUC19RP12	this work[c]
AM1454	$recG_{Q640R}$	P1.AM1432 × AM1418 to Pyr$^+$ (Km^s)
AM1463	$recG_{Q640R}$ Δ*ruvABC*::*cat*	P1.Δ*ruvABC*::*cat* × AM1454 to Cm^r
plasmids		
pGEM-7Zf(−)	Ap^r vector plasmid	Promega
pAM208	pGEM-7Zf(−) *recG*$^+$	Mahdi *et al.* (1997)
pAM331	pGEM-7Zf(−) $recG_{Q640R}$	this work[d]
pJP113	pGEM-7Zf(−) *rdgC*::*dhfr*	J. Peters

[a] All strains are wild-type except as indicated, and are derivatives of MG1655 made by P1 transduction, selecting for the appropriate antibiotic resistance marker.
[b] The Δ*pyrE*::*dhfr* allele was constructed as described (Yu *et al.* 2000). The *dhfr* insertion marking the *pyrE* deletion was amplified from pJP113 and encodes resistance to trimethoprim.
[c] The mutant *recG* allele encoding RecG Q640R was made by changing the glutamine codon CAG to CGG, encoding arginine, using plasmid constructs as described (Mahdi *et al.* 2003). It was cloned into pST76-C (Posfai *et al.* 1997) and introduced into the *E. coli* chromosome of strain MG1655 using the procedures described (Posfai *et al.* 1997), giving AM1432.
[d] A derivative of pAM208 encoding $recG_{Q640R}$ made by restriction fragment replacement as described (Mahdi *et al.* 2003).

region between the branch points of a D loop formed *in vivo* is most likely bound within the RecA filament. Therefore, it is unlikely RecG could translocate this duplex and drive the 5′ branch point into a Holliday junction as suggested previously (Whitby & Lloyd 1995). RecG has been shown to dissociate the invading strand from a D loop structure *in vitro* (McGlynn *et al.* 1997). It could do this by targeting the 5′ branch and translocating the upstream duplex or alternatively by targeting the 3′ branch and translocating the downstream duplex. Both duplex regions should be free of RecA *in vivo*. However, the 5′ branch might be favoured as it resembles a partial replication fork structure with no leading strand at the branch point, the preferred target of RecG *in vitro* (figure 4*a*,*c*; McGlynn & Lloyd 2001*b*).

However, dissociation of a D loop would prevent rescue of a broken replication fork by recombination and might similarly interfere with repair of a DNA DSB generated for example by ionizing radiation. Repair of a DSB relies on D loop formation to extend the 3′ end of a broken duplex. In the classical DSBR model, both ends are engaged by the D loop, resulting in a double Holliday junction intermediate (figure 7; Paques & Haber 1999; Symington 2002). Cleavage of these junctions by a Holliday junction resolvase completes the repair reaction. However, in the SDSA model only one end of the DSB engages. The extended strand is displaced by dissociation of the D loop and anneals with the other DSB end, allowing repair without forming a Holliday junction (figure 7; Paques & Haber 1999; Allers & Lichten 2001; Symington 2002).

6. REPAIR OF IONIZING RADIATION DAMAGE

The inactivation of either RecBCD or RecA recombinase activities confers extreme sensitivity to ionizing radiation (figure 8*a*; Emmerson 1968; Sargentini & Smith 1986), consistent with an essential role in repair of DNA breaks (figure 7). By comparison, loss of RuvABC has a very modest effect in the wild-type MG1655 strain background studied (figure 8*b*,*d*), indicating that Holliday junction resolution is not as essential for survival. The absence of RecG confers a similar modest sensitivity (figure 8*c*). However, the absence of both RecG and Ruv proteins confers extreme sensitivity (figure 8*d*). A similar low survival is seen regardless of whether it is RuvAB, RuvC or both RuvAB and RuvC that is eliminated along with RecG. This is consistent with all three Ruv proteins

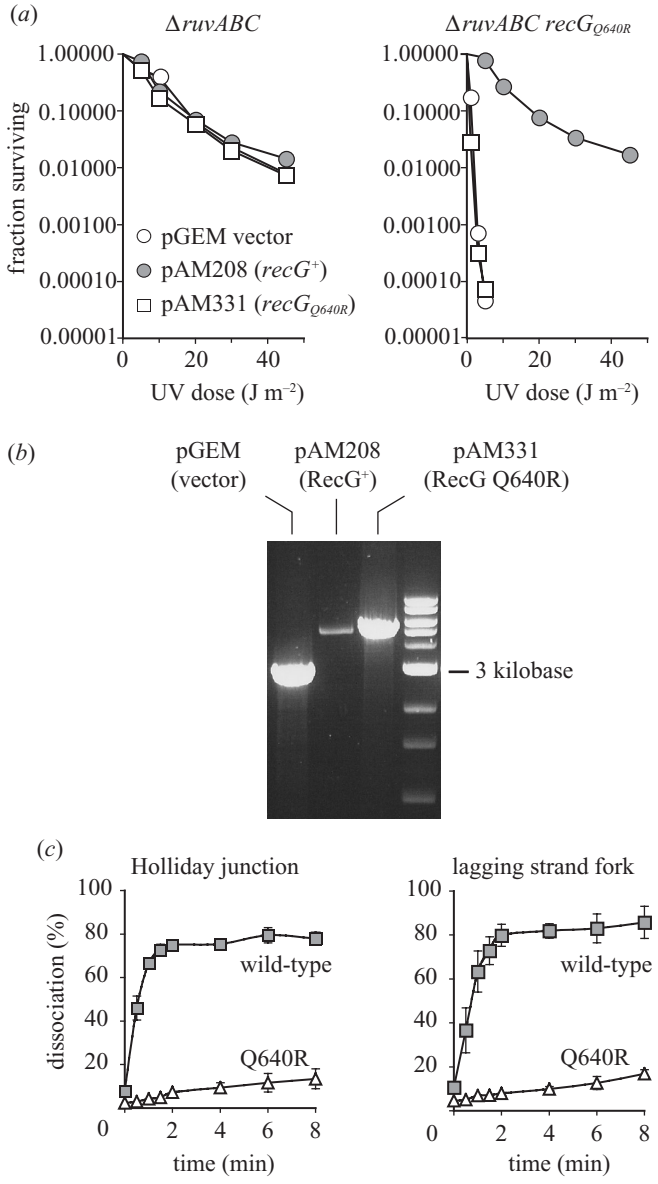

Figure 6. Activity of RecG Q640R. (*a*) Effect of recGQ640R carried either in the chromosome or on a multicopy plasmid on survival of UV-irradiated strains. The strains used, identified by genotype above each panel, were N4583 and AM1463. The plasmids are identified in the left panel and are described in table 1. Sensitivity to killing by UV light was measured as described (Al-Deib *et al.* 1996) using cells grown in LB broth supplemented with ampicillin. (*b*) Effect of RecGQ640R on plasmid copy number. *Escherichia coli* K-12 strain N3793 (*ΔrecG263*::*kan*) was transformed with vector plasmid (pGEM-7Zf(–)), pAM208 (*recG*+) or pAM331 (*recG*$_{Q640R}$) and cultures grown overnight at 37 °C in LB broth supplemented with ampicillin. Plasmid DNA was extracted and samples from equal volumes of culture were digested with *Eco*RI, analysed by agarose gel electrophoresis as described (Mahdi *et al.* 2003). DNA size markers are shown on the right. Note that wild-type RecG reduces plasmid yield quite substantially. (*c*) Rates of unwinding of synthetic Holliday junction and lagging strand fork substrates by RecG wild-type and Q640R proteins. The substrates used and the conditions of the unwinding assays were as described in fig. 5B,C of Mahdi *et al.* (2003). RecG protein was at a final concentration of 100 nM and ^{32}P-labelled substrate DNA at 0.2 nM. Native RecG wild-type and Q640R proteins were purified as described (Mahdi *et al.* 2003).

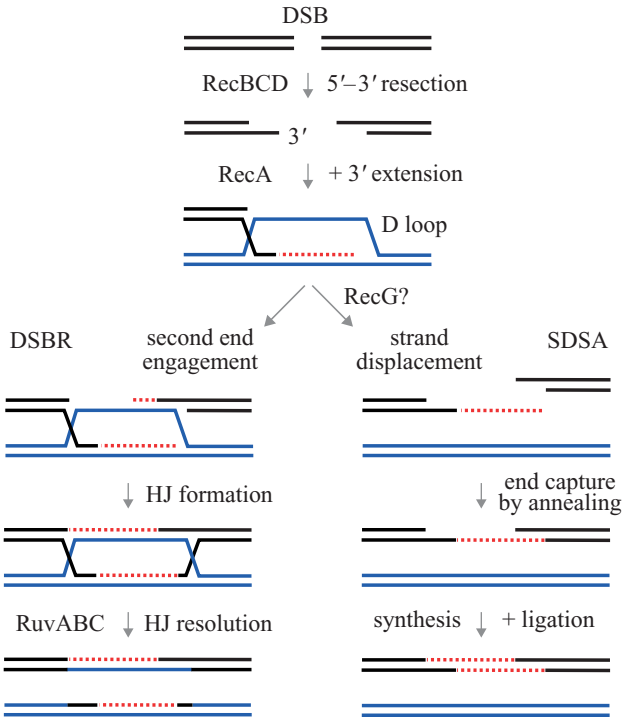

Figure 7. Models for the repair of a DNA double strand break in *Escherichia coli* via formation of a double-Holliday junction (HJ) intermediate (DSBR) or independently of Holliday junctions via synthesis-dependent strand annealing (SDSA model). The DNA ends at the break are resected by RecBCD nuclease to expose 3′ tails on which RecA protein polymerizes, forming a nucleoprotein filament that catalyses homologous pairing and strand exchange with an intact duplex. A single DSB end invades, forming a D loop, which is extended by DNA synthesis. In the DSBR model, the second DSB 3′ end engages the D loop and is extended, resulting in a double Holliday junction intermediate. Resolution of the two junctions in the same or opposite orientation generates a non-crossover (as shown) or crossover product, respectively. In the SDSA model, the extended strand is displaced from the D loop and anneals with the other DSB end. Further synthesis and ligation generates a non-crossover product.

acting as a single complex to resolve Holliday junctions (Mandal *et al.* 1993; Whitby *et al.* 1996; West 1997; Van Gool *et al.* 1998). The extreme sensitivity of the *recG ΔruvC* construct indicates that the DNA branch migration activity of RuvAB cannot substitute for RecG, suggesting that it acts on a different substrate or cannot function without RuvC (Bolt & Lloyd 2002).

Significantly, the inactivation of RecG or RuvABC, or both, has little or no effect on the sensitivity of *recB* or *recA* null strains (figure 8*a–c* and data not shown). Taken together, these data indicate that RecG and RuvABC act in different DSB repair pathways and that both the RecG pathway and the RuvABC pathway depend on RecBCD and RecA. It is tempting to conclude that RuvABC is involved in a DSBR pathway, providing the means to resolve the double Holliday junction intermediate as indicated in figure 7. Likewise, it is tempting to think RecG could promote the SDSA pathway, providing the means to dissociate the D loop intermediate formed by RecBCD and RecA once the 3′ end of the invading strand had been extended (figure 7). In the absence of RecG the D loop

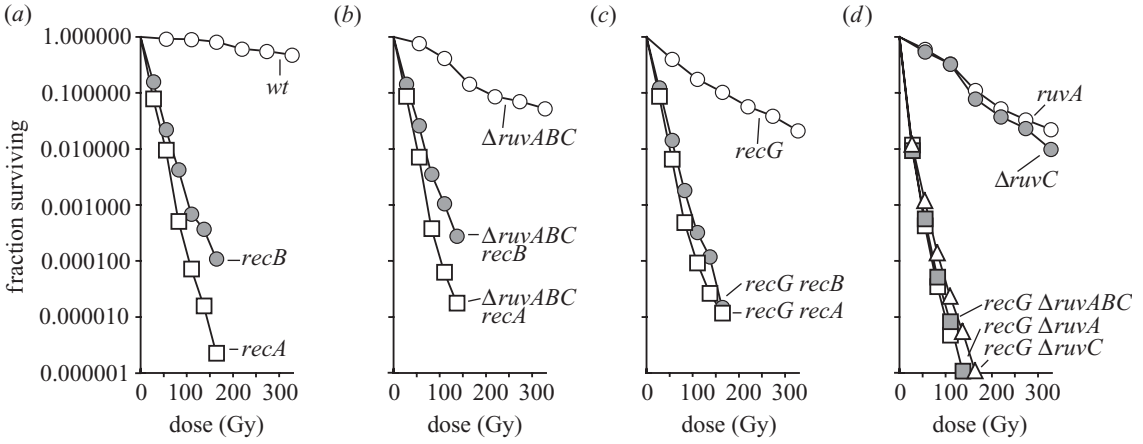

Figure 8. Repair of ionizing radiation damage by the RecBCD, RecA, RecG and RuvABC proteins. The strains used are identified by genotype and listed in table 1, and were (*a*) MG1655, N4278 and N4279; (*b*) N4583, N4851 and N5091; (*c*) N4256, N4702 and N5059; (*d*) N4239, N4259, N4971, N5466 and N5469. Sensitivity to killing by ionizing radiation was measured as described (Al-Deib *et al.* 1996). Irradiated cells were exposed to a ^{137}Cs source at a dose rate of 6.837 Gy min^{-1}.

intermediate would persist and fairly efficient repair could be achieved via the DSBR pathway. In the absence of RuvABC, RecG could divert the intermediates to the SDSA pathway. However, this would not be possible once the double Holliday junction had been ligated. Given ligase is very efficient in *E. coli*, the modest sensitivity of *ruv* mutants therefore suggests intermediates in the DSBR pathway are not ligated at an early stage or that the SDSA pathway predominates. Because it does not involve a Holliday junction intermediate, the SDSA pathway would limit crossing over (Allers & Lichten 2001), which in *E. coli* would result in the formation of circular chromosome dimers. Thus, RecG might reduce the need for XerCD-mediated site-specific recombination to convert dimers to monomers (Michel *et al.* 2000; Barre *et al.* 2001; Sherratt *et al.* 2001), facilitating segregation of repaired chromosomes after exposure to ionizing radiation[1].

7. QUESTIONS AND OUTLOOK

Although recent studies in *E. coli* have shown how interplay between DNA replication, recombination and repair underpin faithful transmission of the genome, several important questions remain unanswered. Is it always necessary to drive a stalled replication fork back from the offending lesion and if so, is repair of the lesion essential *before* restart? Recruitment of a polymerase capable of translesion synthesis might provide an alternative way for replication to resume, although this would risk mutation (Friedberg *et al.* 2002). Such polymerases also lack processivity and are presumably replaced by the normal replicative enzymes once the lesion is passed, but the mechanism facilitating polymerase substitutions are unknown.

What are the factors that do drive fork reversal? RecG, with its ability to translocate a single duplex of a branched DNA molecule, is perhaps the first example of an enzyme engineered to catalyse such a reaction. But current evidence suggests RecG is not the only force available (McGlynn *et al.* 2001; Bolt & Lloyd 2002). Unwinding of the nascent strands by other helicases (e.g. RecQ) and their digestion by exonucleases such as RecJ might allow the parental strands to reanneal (Courcelle & Hanawalt 1999, 2001; Courcelle *et al.* 2003). RecA might provide an alternative when a ssDNA region is exposed at a fork (Robu *et al.* 2001; Lusetti & Cox 2002). Recent studies indicate that RecA together with the RecFOR proteins might stabilize a reversed fork and protect the nascent strands against degradation by RecQ–RecJ activity (Courcelle *et al.* 2003). However, assembling a RecA filament at a damaged fork may also be detrimental in ways that can be prevented by eliminating RecF, RecO or RecR (Moore *et al.* 2003). The RuvAB branch migration complex is another possibility, but is more likely to move forks in the direction opposite to that required for junction formation (McGlynn & Lloyd 2001*a*). It seems better suited to drive fork reversal once a junction has formed, and far enough back to make room for repair. But since RuvAB also underpins junction resolution by RuvC (Mandal *et al.* 1993; Van Gool *et al.* 1998) this raises the question of what prevents breakage of the reversed fork as soon as it meets the sequences targeted by RuvC. How often do reversed forks get broken? This question is very important because once a fork is broken the only way to resume replication is via recombination (figure 2). Direct rescue of a stalled fork has one very important attribute. Replication resumes on the chromosome on which it stalls, and the rescued fork cannot be misplaced. There is no such guarantee with recombination.

Recombination is essential for the repair of a DSB in the DNA. Formation of a D loop by RecA allows for the 3′ end of the broken DNA to be extended. PriA could direct loading of a polymerase for this purpose. However, it might load the entire replisome, initiating both leading and lagging strand synthesis (Xu & Marians 2003). Although this would be required to rescue a broken replication fork, it is not necessarily the most efficient way to repair a DSB, especially given that a RecG-mediated SDSA pathway avoids the complications of crossing over. So, what factors decide the fate of D loops? Genetic and biochemical evidence suggest that the outcome might be determined by RecG and PriA helicase activities and interactions between these two proteins (Al-Deib *et al.* 1996; Gregg *et al.* 2002; Xu & Marians 2003).

What happens to a fork when there is no physical damage to block progression but strand unwinding exposes

direct repeats that might cause slippage, or palindromes that might form hairpin loops? Such events appear to cause deletions or duplications, provoke recombination or induce DNA breakage (Leach 1994; Morag *et al*. 1999; Saveson & Lovett 1999; Cromie & Leach 2000; Bzymek & Lovett 2001*a*,*b*). However, we still know very little of the molecular details.

How applicable are the bacterial models to other organisms? There is no homologue of RecG in eukaryotes other than in the mitochondria of certain plants. However, studies of the RecQ family of DNA helicases and of their interactions with the recently identified RuvABC-like Holliday junction resolvase and of the Mms4-Mus81 endonuclease (Chakraverty & Hickson 1999; Boddy *et al.* 2001; Kaliraman *et al.* 2001; Myung *et al.* 2001; Constantinou *et al.* 2002; Doe *et al.* 2002) suggest that the interconversion of replication fork and Holliday junction structures may play a pivotal role in protecting the genome in eukaryotes, including humans. DNA structures consistent with this possibility have been identified recently in yeast (Sogo *et al.* 2002). The human RecQ helicase family members BLM and WRN unwind a variety of branched DNA structures, including Holliday junctions (Constantinou *et al.* 2000; Karow *et al.* 2000). The fission yeast RecQ homologue Rqh1 may catalyse removal of Holliday junctions formed from a fork by unwinding them back to forks (Doe *et al.* 2002), whereas the budding yeast homologue Sgs1 may do the opposite (Kaliraman *et al.* 2001). Thus, it seems that replicating cells in all organisms may have to strike a balance between avoiding collapse of replication forks to reduce harmful rearrangements and provoking their collapse to bypass lesions and replicate their DNA.

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ENDNOTE

[1]Recent studies have shown that *ruv xerC* mutants of *E. coli* are much more sensitive to ionizing radiation than a *ruv* single mutant (T. R. Meddows, Andrew P. Savory and R. G. Lloyd, unpublished data). Since the double mutant retains RecG protein but lacks the XerCD site-specific recombination activity needed to resolve chromosome dimers formed by crossing over between sisters into monomers, this observation indicates that the RecG pathway for DSB repair promotes crossing over rather than avoiding it, which implies that repair is not achieved *via* SDSA.

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GLOSSARY

DSB: double-strand break
DSBR: DSB repair
dsDNA: double-stranded DNA
SDSA: synthesis-dependent strand annealing