Initiation of genetic recombination and recombination-dependent replication

Stephen C. Kowalczykowski

Recombination initiates at double-stranded DNA breaks and at single-stranded DNA gaps. These DNA strand discontinuities can arise from DNA-damaging agents and from normal DNA replication when the DNA polymerase encounters an imperfection in the DNA template or another protein. The machinery of homologous recombination acts at these breaks and gaps to promote the events that result in gene recombination, as well as the reattachment of detached replication arms and the resumption of DNA replication. In *Escherichia coli*, these events require collaboration (RecA, RecBCD, RecFOR, RecQ, RuvABC and SSB proteins) and DNA replication (PriABC proteins and the DNA polymerases). The initial steps common to these recombination and recombination-dependent replication processes are reviewed.

AS POINTED OUT in the Editorial that leads off this Special Issue of *TiBS*, the three R's of genome maintenance and perpetuation are all interconnected. In this introductory article, the historical antecedents of this merger of DNA recombination, replication and (in part) repair are developed, followed by an overview of the interactions that comprise the recombination–replication interface as we understand it today. I hope that this summary will serve as a framework to integrate the more specialized articles about various specific aspects of the DNA recombination–replication interface that follow. This article concludes with a speculative discussion of the biological and evolutionary considerations that bind recombination, recombination-dependent replication and the repair of DNA breaks within a common mechanism.

A DNA break that remains unrepaired is lethal for any cell or its progeny. Double-stranded DNA (dsDNA) breaks (DSBs) can be produced directly by ionizing radiation, such as γ rays, and indirectly as a natural consequence of DNA replication on a chemically flawed template (Fig. 1). If, during DNA replication, the leading-strand DNA template were to contain just a simple nick, then a blunt DSB would result; if the lagging-strand template were to have a nick, then a DSB with a 3′-single-stranded DNA (ssDNA) tail would result. Finally, replication of a linear chromosome produces a DSB with a 3′-ssDNA tail that somehow must be replicated or 'repaired' (see articles by K. Kreuzer and by A. Kass-Eisler and C. Greider in this issue of *TiBS*). Similarly, ssDNA gaps (SSGs) can arise from the repair of exogenous DNA damage, as well as from blocked DNA replication.

Recently, there has been a growing appreciation that, in the absence of exogenous DNA-damaging agents, most DSBs and SSGs result from the encounter of a replication fork with 'normal' discontinuities in the template that derive from an endogenous source (e.g. nicks caused by free radicals arising from intermediary metabolism); the inferred frequency of such roadblocks to DNA replication in *E. coli* can approach one per cell division. On the other hand, DSBs and SSGs are canonical sites for initiation of homologous recombination. It might seem paradoxical that potentially lethal DNA lesions are needed for a universal biological process such as genetic recombination. However, it has now become clear that recombination is important not only for repairing such lesions but also for re-initiating the replication process. In fact, recombination repairs not only the DNA-strand breaks, but it also rectifies the aborted DNA replication process itself. Thus, recombination is an important component of DNA replication, and both its ubiquity and its evolutionary conservation would, in fact, argue that it is an essential component.

S. C. Kowalczykowski is in the Sections of Microbiology and of Molecular and Cellular Biology, University of California, Davis, CA 95616, USA.
Email: sckowalczykowski@ucdavis.edu

A brief history

Although the fields of DNA replication and recombination progressed more or less independently, early in the development of each field, prescient individuals appreciated their interconnections. Classical recombination models, called 'break-copy' or 'copy-choice', recognized replication (the 'copy' part of each model's name), as an explicit component of recombination[1,2]. Subsequent models, such as the Meselson-Radding or DSB repair model (Fig. 2), invoked limited replication as an essential element of the recombination process. However, in each model, the role of replication was only to complete the recombination event, and the extent of replication was limited; furthermore, it was evident from phage studies that replication was not essential to the recombination process.

Similarly, elaboration of DNA replication in *E. coli* developed almost independently of the study of genetic recombination. Given both the apparent obligate need for origin (*oriC*)-dependent initiation and the high processivity of the DNA polymerase III (Pol III) holoenzyme system, it was presumed that, once initiated at the origin, most replication forks would proceed to completion at the replication terminus[3]. However, a few studies suggested that replication forks might actually encounter some type of 'barrier', which could cause dissociation of some or all of the replisome prior to complete replication of the chromosome. This would result in the 'collapse' of a replication fork[4]. If replication were unable to reinitiate in an *oriC*-independent manner near the point of collapse, then such a collapse would be lethal to one of the daughter cells. To restart replication, the Pol III holoenzyme would need to be reassembled, but in some cases the resultant DSB or SSG would also need to be repaired. A solution to this cellular calamity was actually hypothesized decades ago (e.g. Ref. 5 and Chapter 1 in Ref. 2): it involved 'repair' of the detached replication arm by homologous recombination[6,7]. A central step of recombination is the invasion of a homologous duplex by ssDNA to form a structure known as 'displacement loop' (D-loop); DNA strand invasion by the 3′end of ssDNA allows it to serve as a potential primer for DNA replication. This idea was confirmed by the pioneering

0968–0004/00/$ – See front matter PII: S0968-0004(00)01569-3

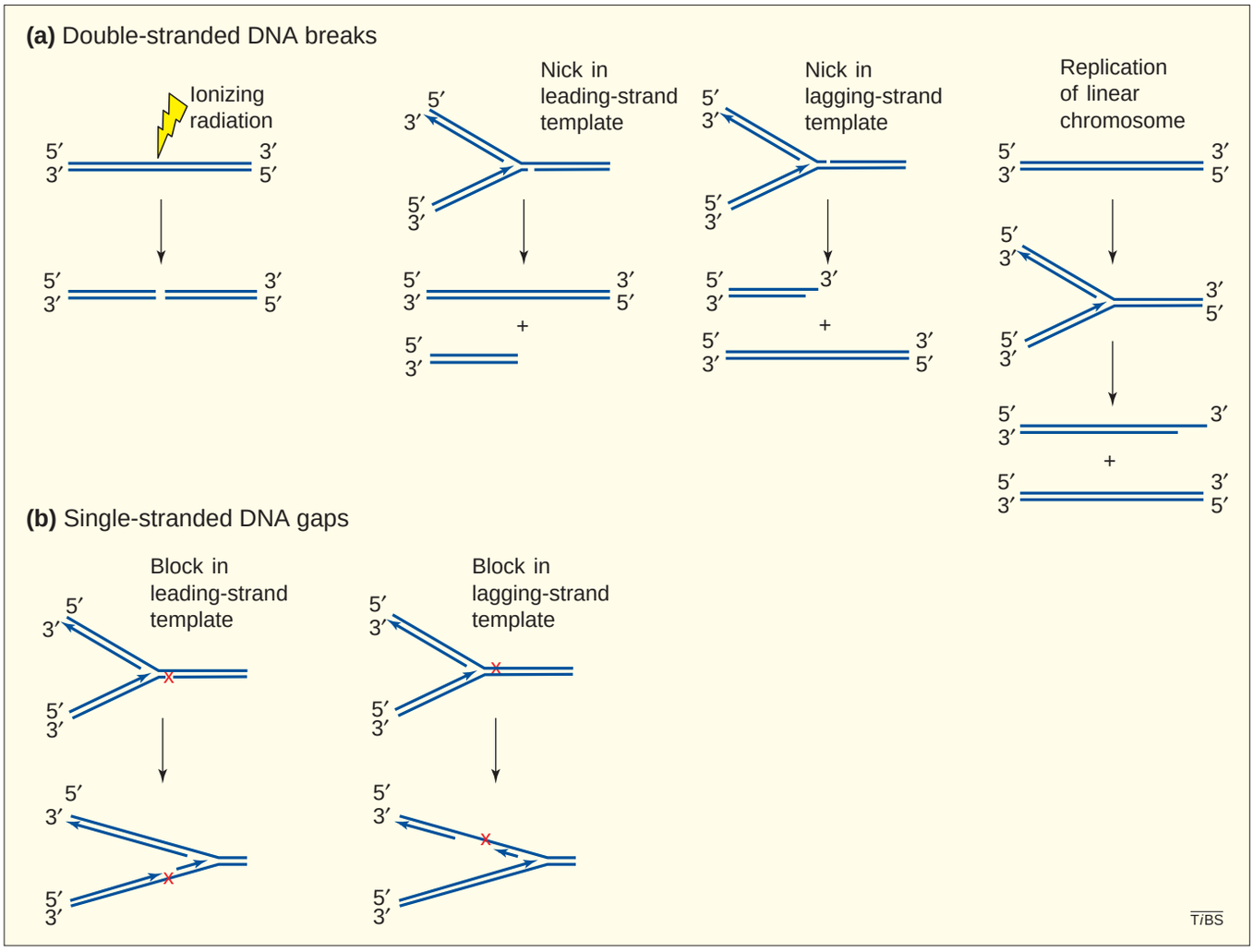

Figure 1

Generation of double-stranded DNA breaks (DSBs) and single-stranded DNA gaps (SSGs). **(a)** DSBs can be created by a variety of means; these include (from left to right): ionizing radiation; DNA polymerase encountering a nick in the leading-strand template; DNA polymerase encountering a nick in the lagging-strand template (note that the DSB could have a 3′-ssDNA overhang); and replication of a linear chromosome in the absence of a mechanism to complete synthesis of the lagging strand. **(b)** SSGs can be created when DNA polymerase encounters an impediment in the leading-strand template, but synthesis ensues further downstream, or when the polymerase encounters an impediment in the lagging-strand template. Nicking the SSG will convert it into a DSB.

studies of bacteriophage T4 replication, where recombination-dependent replication was shown to be an essential part of T4 late replication[8]. However, despite the elegance of the work, its extension to other organisms remained underappreciated (see article by K. Kreuzer in this issue of *TiBS*).

More recently, the seminal experiments by Kogoma and colleagues refocused attention on the crucial interplay between replication and recombination[6,9,10]. Kogoma's laboratory established that, in the absence of canonical origin function, normal levels of cellular replication could indeed occur, but with some notably unique characteristics[11]. One process, called induced-stable DNA replication (iSDR), required RecA, RecBCD, the recombination hotspot χ and PriA proteins, and initiated at D-loops (structures in which ssDNA is base-paired to one strand of dsDNA and, as a result, the complementary strand of the dsDNA is displaced; see Figs 2,3). Another process, called constitutive-stable DNA replication (cSDR), required RecA and PriA protein, and initiated at R-loops (structures in which an RNA strand is base-paired to one strand of dsDNA, and the complementary DNA strand is displaced). Thus, both processes required the function of enzymes that were essential to early steps of recombination for this *oriC*-independent mode of DNA replication. Despite the absence of normal origin function, these cells were viable[6,12], although replication initiation occurred randomly, disregarding cell cycle or chromosomal location.

Kogoma summarized these observations under the name of 'recombination-dependent replication' (RDR)[6]. He suggested that this mechanism for starting DNA replication *de novo* could serve as an ideal means of reinitiating DNA replication when the replication fork was disrupted for whatever reason. Thus, recombination was the solution for the problem of reinitiating replication in the absence of a defined origin. In parallel, Kuzminov and co-workers reconciled many genetic, biochemical and physiological phenomena by proposing that RDR functioned in almost every cell cycle to reattach collapsed replication forks that arose from the unavoidable basal level of DNA damage (e.g. nicks) that exists in all DNA (Refs 2,4,7). Simple, but not previously fully understood, phenotypes such as the high lethality (50–70%) of *recA* or *recB* (or *recC*) mutants could be readily explained by the simple fact that at least 50% of the cells encountered at least one lesion, which caused replication fork collapse and required recombination

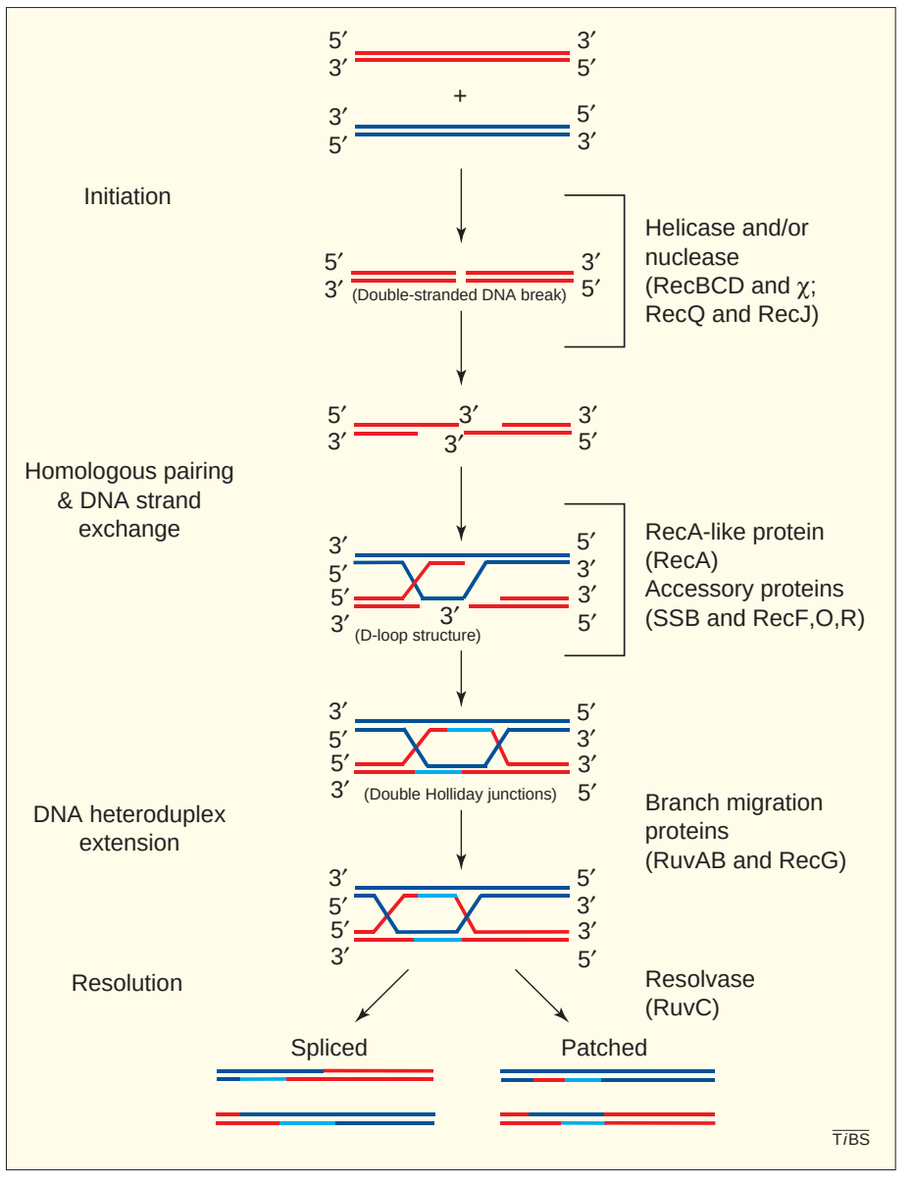

Figure 2

General model for homologous recombination. The model depicted is the double-strand-break repair (DSBR) model[18]. This model, in its most general form, applies to homologous recombination in all organisms; the first two steps of this mechanism also apply to initiation of RDR. The text in parentheses designates the proteins from *E. coli* that function at the indicated step. Light-blue lines indicate newly synthesized DNA. Adapted from Ref. 20.

function for resumption. The increased number of nicks in *polA* (DNA Pol I) mutants explained the inviability of *polA recA* double mutants.

The connection of recombination to replication was made more explicit when a function for one of the more perplexing replication proteins, PriA, in the resumption of disrupted DNA replication was proposed[13] (see article by K. Marians in this issue of *TiBS*). Subsequently, PriA protein was discovered to be required for both RDR (Ref. 14) and genetic recombination[15]. This important discovery brought the inter-relationship between DNA replication and homologous recombination to closure: the PriA protein is essential both for the replication that requires recombination, and for the recombination that requires replication.

Homologous recombination in *E. coli*: an overview

At least 25 different proteins are involved in all types of homologous recombination in *E. coli*[16]; these include the RecA, RecBCD, RecF, RecG, RecJ, RecN, RecO, RecQ, RecR, RuvAB, RuvC, PriA and SSB proteins, DNA polymerases, DNA topoisomerases and DNA ligase, as well as the *cis*-acting recombination hotspot χ. Many of these proteins have functional (although not necessarily structural) homologs in Bacteria, Eucarya, Archaea and some phages; in fact, a RecA-like protein is present in all free-living organisms examined so far[17].

DSBs serve the paradoxical role of being both sites at which recombination initiates and lesions that are lethal; hence, they are simultaneously loci that stimulate recombination and DNA damage that needs repair. For this reason, a model for recombination and the repair of dsDNA breaks is called the double-strand-break repair (DSBR) model[18]. The recombinational repair process consists of four steps: (1) initiation (processing), (2) homologous pairing and DNA strand exchange, (3) DNA heteroduplex extension (branch migration) and (4) resolution (Fig. 2).

The first step, initiation, represents a processing of the linear duplex DNA at the DSB to produce the ssDNA needed for DNA strand invasion of a dsDNA homolog by RecA protein. For the prototypic homologous pairing reactions promoted by RecA protein, ssDNA is a prerequisite[16,19–21] (but see Refs 22,23 for notable exceptions). For the second step of recombination, DNA strand exchange, to occur between two homologous dsDNA molecules, processing of one duplex to produce a region of ssDNA is conventionally invoked. This processing involves the recombination-specific helicases, the RecBCD and RecQ proteins, with the latter thought to work in conjunction with the RecJ exonuclease. RecBCD enzyme is a DNA helicase that also possesses a nuclease activity that is regulated by the recombination hotspot χ (Ref. 24). Modification of RecBCD enzymatic activity by χ is coordinated with the loading of RecA protein onto the χ-containing ssDNA by RecBCD enzyme, ensuring incorporation of this ssDNA into a recombinationally proficient nucleoprotein complex. RecQ protein is also a DNA helicase; in wild-type cells it functions in the so-called RecF pathway, which functions predominantly at SSGs, but it can also act efficiently at DSBs when the RecBCD is rendered non-functional by mutation (and when suppressor mutations, *sbcBC*, are present)[25]. If RecA protein fails to assemble on the ssDNA produced, then accessory proteins RecF, RecO and RecR facilitate this assembly step[26,27]. Upon assembly of a contiguous RecA protein filament on ssDNA, called the presynaptic filament, the subsequent homology search can ensue. The third step of recombination is DNA heteroduplex extension; here, a specialized motor protein complex, the RuvAB complex, functions. The RuvAB complex is a DNA helicase

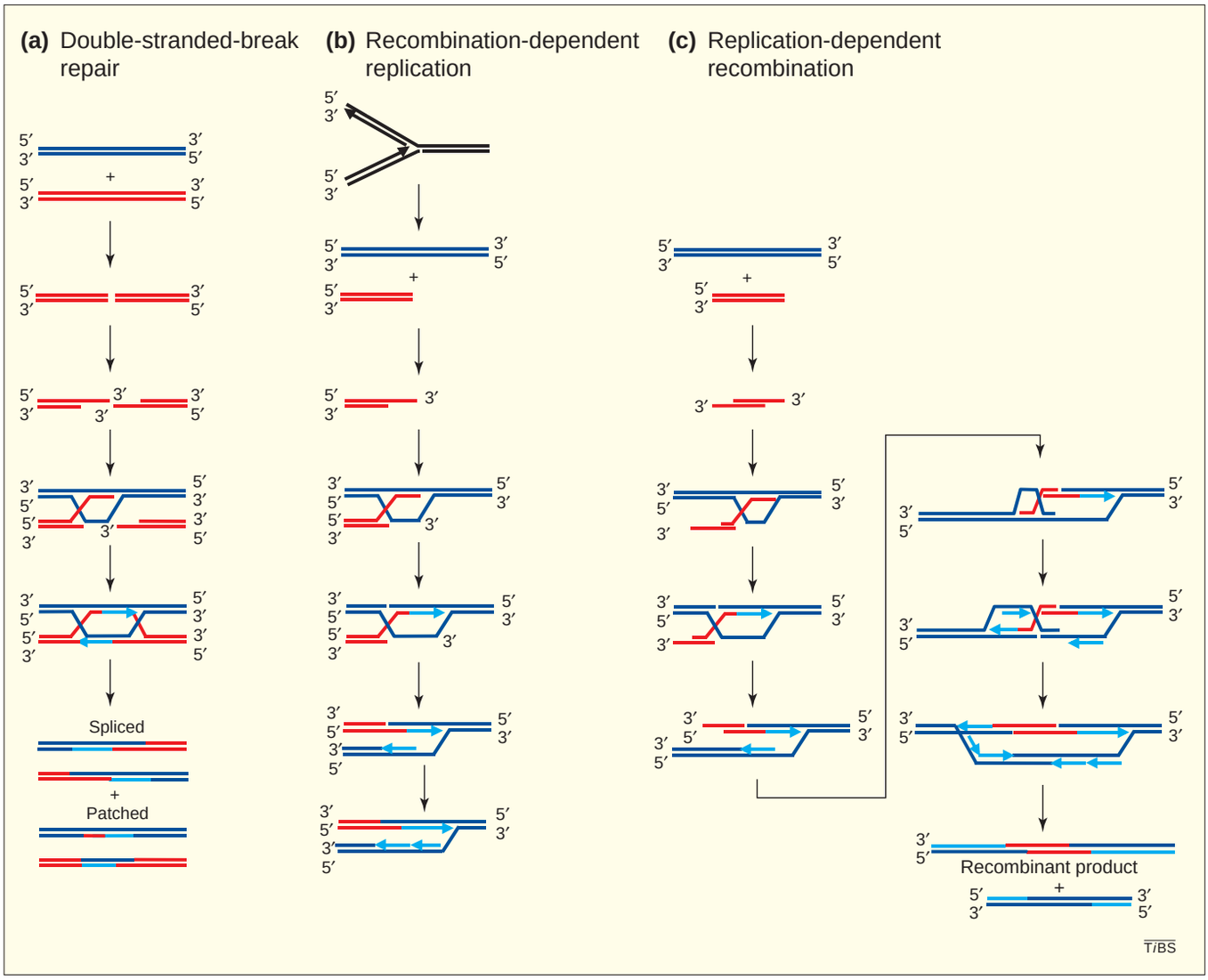

Figure 3

Biochemical models for double-strand-break (DSB) repair, recombination-dependent replication and replication-dependent recombination. Light-blue lines indicate newly synthesized DNA. **(a)** Recombination resulting from the repair of a DSB; invasion of the intact dsDNA homolog by each of the processed 3′-ssDNA ends need not occur simultaneously. Modified after Ref. 18. **(b)** A nick in the leading-strand template generates a DSB; if the nick were in the lagging-strand template (not shown), then it is possible that a DSB with a 3′-ssDNA tail would be generated that did not require further processing. **(c)** Integration of a linear DNA fragment (red) by conjugation (or transduction) into (a region of) chromosomal DNA (blue); processing of the dsDNA fragment is shown as occurring in one step, but those processing events need not be simultaneous.

that extends the region of DNA heteroduplex by branch migrating the crossover point[28]. The final step of recombination requires separation of the two conjoined DNA molecules. This important resolution step is left to a Holliday junction-specific endonuclease, the RuvC protein. The RuvC protein, as part of a complex with the RuvAB proteins, recognizes and cleaves Holliday junctions to complete the recombination process[29].

The ordered completion of these steps results in formation of recombinant progeny that display the classical genetic hallmarks of crossing-over or gene conversion. However, a simple alteration of how the homologously paired DNA is used or how the Holliday junction is processed, allows these recombination intermediates to be converted into substrates for replication (Fig. 3).

Initiation of recombination: RecBCD and RecQ helicases

The RecBCD enzyme is essential for 99% of the recombination events occurring at DSBs in wild-type *E. coli*[16,24]. First identified as an essential enzyme for homologous recombination[30], RecBCD is also important in the repair of DNA damage and dsDNA breaks[31], the reattachment of detached arms of replication forks[4], the initiation of replication in the absence of normal *oriC* function[12,32], and the 'adaptive' response of stressed bacteria[33]. Yet, incongruously, it is also responsible for >90% of the degradation of foreign DNA.

RecBCD enzyme possesses a number of seemingly disparate enzymatic activities, including ssDNA exonuclease, ssDNA endonuclease, dsDNA exonuclease, DNA-dependent ATPase and DNA helicase activities[16]. This helicase shows a marked preference for blunt or nearly blunt dsDNA ends, further distinguishing it from other helicases, which typically prefer DNA substrates with ssDNA tails. This skein of activities is easily understood if one simply views the RecBCD enzyme as a DNA helicase with an associated ssDNA endonuclease activity: it unwinds dsDNA and, simultaneously, endonucleolytically cleaves the ssDNA that it produces (Fig. 4). RecBCD enzyme binds tightly to the end of a dsDNA substrate (K_m~1 nM)[34], unwinds it at

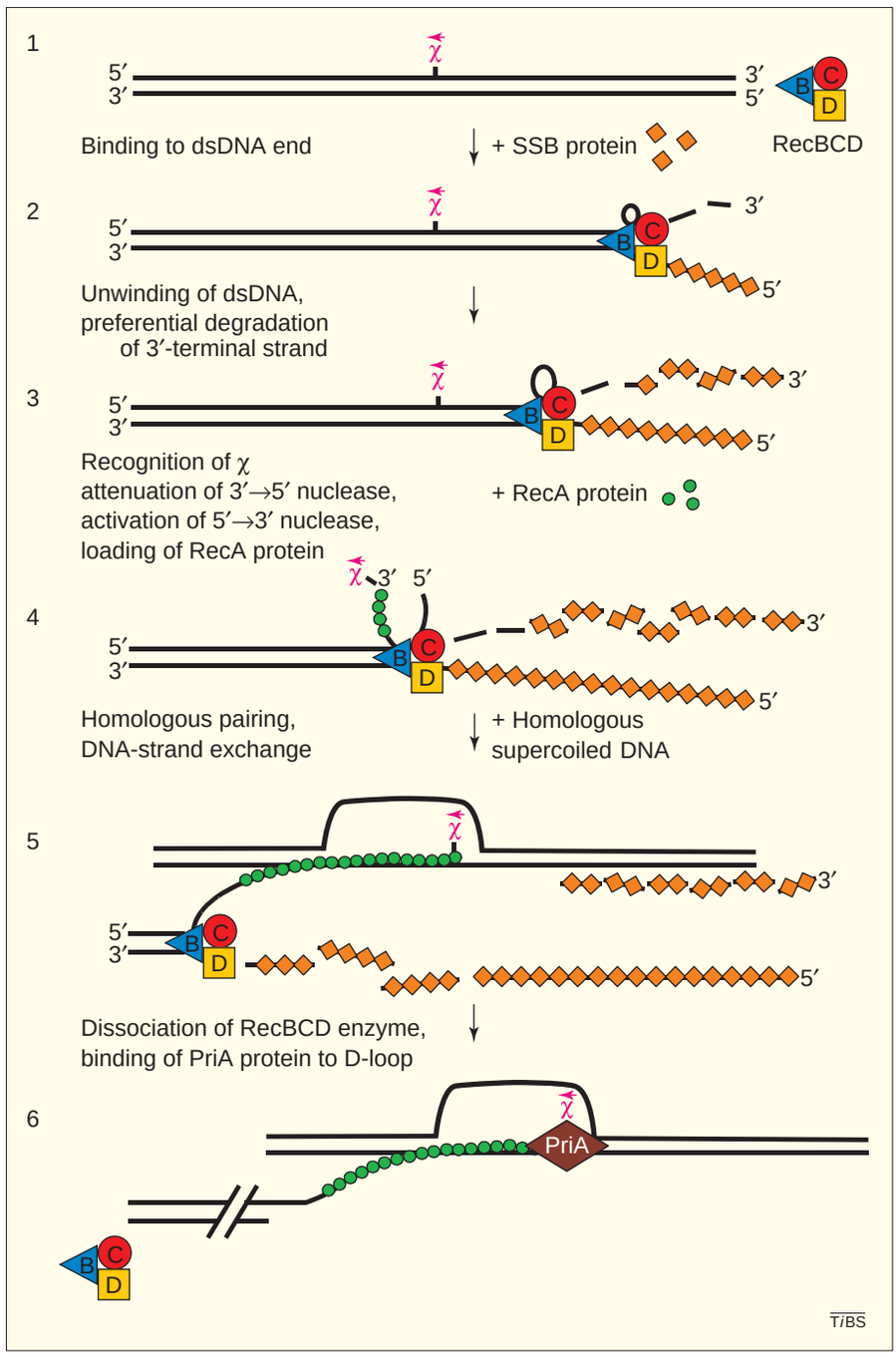

Figure 4

Early steps of homologous recombination and recombination-dependent repair (RDR) in *E. coli* are coordinated by RecA protein, RecBCD enzyme and χ. RecBCD enzyme binds to the end of a DSB. It unwinds the dsDNA while preferentially degrading the strand that was 3′-terminal at the entry point. Interaction with χ results in attenuation of the 3′→5′ nuclease activity; activation of a weaker 5′→3′ nuclease activity; and the facilitated loading of RecA protein onto the χ-containing ssDNA that was produced by continued DNA unwinding beyond χ. The resulting RecA protein–ssDNA filament invades homologous dsDNA, to produce a D-loop structure. After formation of the D-loop, recombination could either proceed as depicted in either Fig. 3a, or PriA protein could bind to initiate RDR as shown in Fig. 3b or c. Adapted from Ref. 24.

approximately 1000 bp/sec, translocates for about 30 000 bp before dissociating, and consumes 2–3 ATP molecules/bp unwound[34–36]. During translocation, it produces growing 'loop-tail' or 'twin-loop' structures[37].

The most remarkable feature of RecBCD enzyme action is the way in which the recombination hotspot χ regulates its biochemical activities. Upon recognizing the χ sequence, the 3′→5′ nuclease activity is attenuated, whereas a weaker 5′→3′ activity is activated and its helicase activity remains unaltered[38–40]. Thus, χ not only attenuates the overall nucleolytic activity of RecBCD enzyme, but it also switches the polarity of DNA-strand degradation. The consequence of these biochemical changes is the generation of ssDNA with χ at its 3′ terminus[41], which is the end that is optimal for RecA-protein-dependent DNA strand invasion of dsDNA and needed to prime DNA replication (Fig. 4). However, the assembly of RecA protein onto that processed ssDNA is not left to chance. Following interaction with χ, RecBCD directs the loading of RecA protein onto the χ-containing ssDNA, to the exclusion of the inhibitory SSB protein, thereby contributing to the heightened invasiveness of that ssDNA (Refs 38,42). This loading is an essential biological function of the RecBCD–χ interaction[43]. The molecular event responsible for these changes in RecBCD enzyme is unknown, but the model that the RecD subunit is modified or inactivated at χ is consistent with many genetic and biochemical observations[44]. The recombination-proficient RecBC enzyme (without the RecD subunit) is a helicase with little or no nuclease activity[45] that retains the ability to load RecA protein asymmetrically onto ssDNA (onto the strand 3′-terminated at the entry site); loading by RecBC enzyme is constitutive and independent of χ (Ref. 46).

Inactivation of RecBCD enzyme results in cells that recombine poorly and display decreased viability (~30%). However, *E. coli* has another path for homologous recombination, called the RecF pathway. When RecBCD enzyme is rendered nonfunctional, recombination can proceed at nearly wild-type levels in strains with *sbcB* (exonuclease I) and *sbcC* (or *D*) mutations[47]. Approximately 75% of the events require *recQ* function, and the remaining 25% use *uvrD* and *helD* functions[48]. Like the RecBCD enzyme, RecQ protein is a recombination-specific helicase[49], without nuclease activity, and unwinds plasmid-sized DNA in the presence of SSB protein[50]. However, it does not load RecA protein onto ssDNA (F. Harmon and S.C.K., unpublished), which poses a problem for RecA protein, because it must displace the SSB protein from the unwound ssDNA. RecQ helicase works in conjunction with RecF, RecO and RecR, and the latter two proteins facilitate the displacement of SSB protein from ssDNA and enable its replacement with RecA protein[26,27].

Recombination hotspots: χ sites

The recombination hotspot χ was originally discovered as a *cis*-acting mutation in bacteriophage λ that conferred better growth in *E. coli*[51]. Since that

discovery, it has become abundantly clear that χ is an essential component of the recombination process in *E. coli*, with χ homologs providing a similar functionality in other bacteria[52–54]. The χ sequence is a recombination hotspot because it modifies the enzymatic activities of RecBCD enzyme. For decades, the vigorous nucleolytic activity of RecBCD enzyme posed a dilemma for those trying to reconcile its potent degradative activity, with its essential role in many diverse biological processes. This paradox was resolved with the discovery that χ regulates the activity of RecBCD enzyme[38–40,55]. The asymmetric χ sequence is recognized only from within dsDNA by the translocating RecBCD enzyme, and the 5′-GCTGGTGG-3′ sequence on the DNA strand that is 3′-terminated at the dsDNA entry site for RecBCD enzyme is sufficient to attenuate its 3′→5′ nuclease activity[56]. As a consequence, DNA downstream of χ is protected. However, because the helicase activity is unaffected, ssDNA is produced for RecA-protein-mediated strand invasion[38,39] and, in fact, RecA protein is loaded onto this DNA strand by the translocating χ-modified RecBCD enzyme[42]. Thus, χ is a polar hotspot *in vivo*[51] because, relative to the DNA upstream of χ, the downstream χ-containing ssDNA is both preserved and complexed with RecA protein[43].

The biological importance of χ was underscored when the sequence of the *E. coli* genome was completed. There are 1009 χ sites in the genome, a value which is 4–8-fold higher than randomly expected. Thus, *E. coli* is willing to pay the entropic cost of maintaining an over-represented supply of χ sequences[57]. Furthermore, the χ sequence, being asymmetric, is not oriented randomly in the genome: about two-thirds of the χ sequences are oriented so that χ 'points' back to the origin (Fig. 5). The significance of both the over-representation and the non-random orientation of χ was appreciated by Kuzminov[58], when he suggested that both features would be important if the collapsed replication forks were to be repaired by a RecBCD enzyme-dependent recombination event, which, in turn, required χ to become activated for recombination. If a replication arm were to detach because of a nick on either the leading strand or lagging strand template, then the subsequent re-attachment of the broken arm with the sister duplex could occur by RecBCD-dependent, χ-dependent recombination. Thus, χ serves a biological function that both includes and transcends its role as a 'recombination hotspot' by maintaining the integrity of the replicating chromosome. Serving such an indispensable function readily explains its over-representation in the *E. coli* genome. Recent studies of χ homologs in other bacteria confirm these ideas: although those sequences are different from the canonical *E. coli* χ sequence, they are over-represented in their respective genomes[52], and they elicit the same biological and biochemical changes in their cognate RecBCD-like enzymes[53,54].

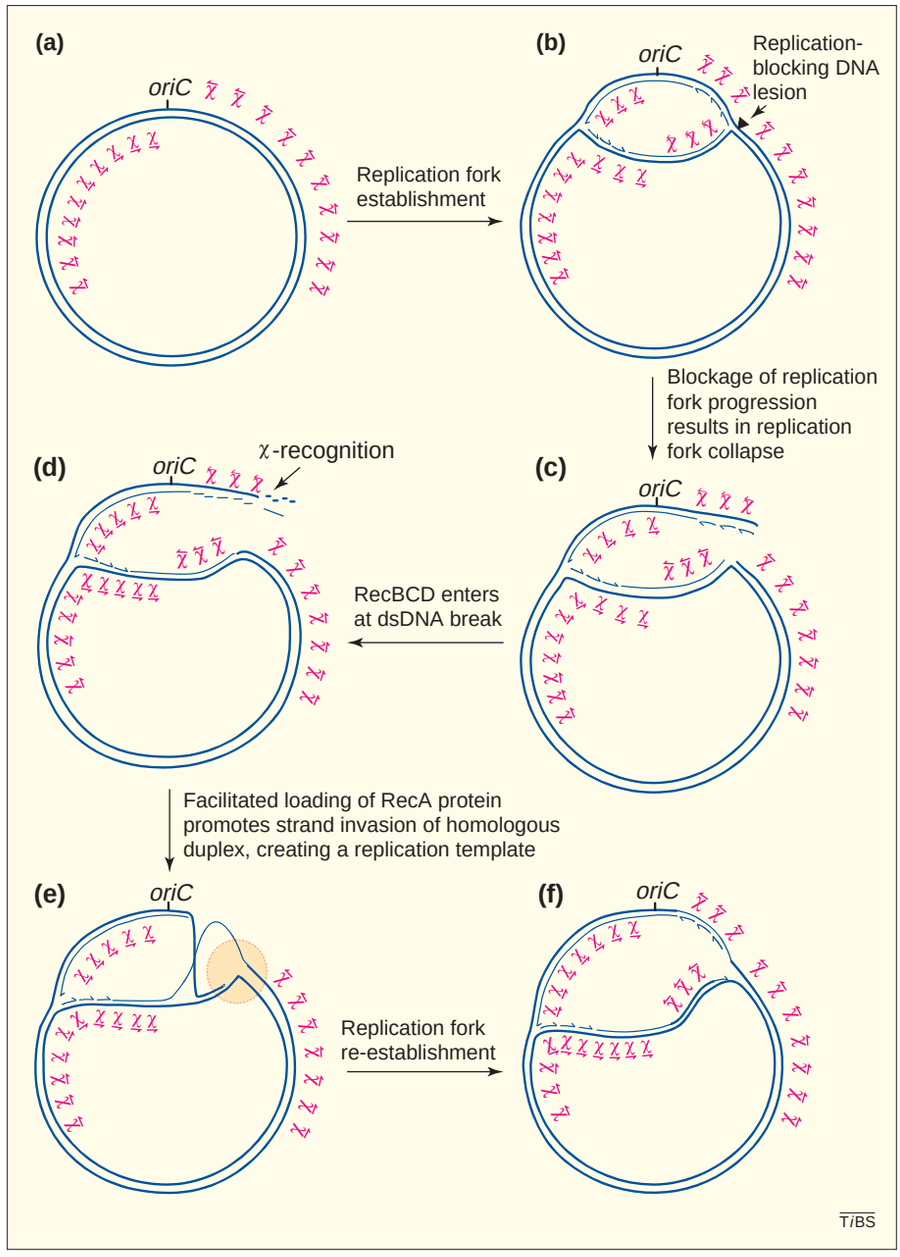

Figure 5

Recombinational repair of a collapsed replication fork. **(a)** The unreplicated *E. coli* chromosome with an exaggerated view of the asymmetric distribution of χ sites around the origin. **(b)** Replication occurs bi-directionally, initiating at *oriC*; a lesion in the DNA template blocks progression of one replication fork. **(c)** The blockage causes detachment of one arm and collapse of the replication fork, resulting in a DSB. **(d)** RecBCD enzyme enters at the dsDNA end, degrading until reaching a χ site; enzymatic modification of RecBCD enzyme occurs and the facilitated loading of RecA protein follows. **(e)** RecA protein promotes strand invasion of the χ-containing ssDNA into the homologous duplex, recreating a substrate for PriA-dependent re-assembly of the Pol III holoenzyme (shaded circle). **(f)** Replication of the chromosome resumes. Adapted from Ref. 24.

Homologous pairing and DNA strand exchange: RecA, SSB, RecF, RecO and RecR proteins

The RecA protein is required for nearly all homologous recombination in *E. coli*[20]. It possesses ATPase, coprotease, DNA renaturation and DNA-strand exchange activities[17,19,21]. RecA-promoted DNA strand exchange involves a number of

kinetically distinct steps: presynapsis, the formation of a functional RecA-protein–ssDNA complex; synapsis, the search for and pairing of homologous DNA; joint molecule formation, the nascent exchange of DNA strands; and branch migration, the polar exchange of DNA strands.

The specific mechanism by which RecA protein finds DNA sequence homology is unclear; it is known, however, that neither the homology search nor the formation of up to several kilobase pairs of heteroduplex DNA require ATP hydrolysis[59]. Thus, one could view the presynaptic filament as an extended sequence-specific DNA-binding protein that finds homology in dsDNA by using the same kinetic search mechanisms available to other sequence-specific DNA-binding proteins, such as the Lac repressor: the entire search process must be passive, involving nothing more than one- and three-dimensional diffusion processes[60]. Compared with a sequence-specific-binding protein, however, the presynaptic complex is huge and sequence specificity is conferred by the sequence of the ssDNA bound within the filament and not by the protein itself. Also, the DNA-strand exchange process does not utilize directly the energy derived from ATP hydrolysis; the free energy of binding of the presynaptic complex to the homologous dsDNA is sufficient to promote exchange, and ATP hydrolysis serves largely to dissociate the complex and to re-set the catalytic cycle[59].

Although the binding of RecA protein to ssDNA is generally sequence independent, *in vitro* selection experiments revealed that RecA protein (and its homologs) displays a preference for GT-rich ssDNA (Ref. 61). But perhaps more surprising, the selected ssDNA is a better substrate for RecA-protein-promoted DNA strand invasion (D-loop formation). Even though RecA protein binds more tightly to these selected sequences than to control sequences, the pairing result is unexpected because the rate-limiting step in D-loop formation is not the ssDNA binding step, but rather the dsDNA 'opening' step[62]. Hence, it appears that amongst the RecA family of proteins, there was selective pressure to preferentially bind sequences that are also preferred DNA pairing sequences. Interestingly, the χ sequence is a member of these preferred binding/pairing sequences[61]. This result might not be a coincidence; instead it seems to be a very logical bias that is readily understood in the context of RDR (see below).

Recent studies have expanded the repertoire of DNA- (and RNA-)strand exchange processes that RecA protein can promote. In the canonical DNA-strand exchange reaction, RecA protein assembles on ssDNA (via a primary DNA-binding site), and this nucleoprotein complex interacts with homologous dsDNA (via a secondary DNA-binding site) to promote DNA strand exchange 'within' the confines of this complex (i.e. *in cis*). However, RecA protein can also mediate an exchange reaction between DNA molecules when neither is bound at the primary site within the protein filament (i.e. *in trans*), suggesting that the RecA protein filament serves as a catalytic surface to 'activate' the normally stable dsDNA for pairing with ssDNA, and affording insight into how a reaction as complex as DNA strand exchange might have evolved[22]. RecA protein can also initiate an exchange reaction when assembled as a dsDNA presynaptic filament, rather than the conventional ssDNA filament[23]. This 'inverse' DNA-strand exchange reaction could contribute to the second asymmetric DNA-strand exchange event of DSBR (Fig. 2). In addition, the RecA-protein–dsDNA complex can promote an inverse RNA-strand exchange reaction with ssDNA to produce an RNA–DNA hybrid (or R-loop)[23,63]. This reaction is particularly interesting with regard to RDR, because such functionality for RecA protein was proposed by Kogoma to be an essential step of this mechanism[10] (see above).

DNA strand exchange mediated by RecA protein can be stimulated by an ssDNA-binding (SSB) protein, provided that the SSB protein does not complex with the ssDNA before RecA (Ref. 64). SSB protein is a prototypic representative of a class of ssDNA-binding proteins that have no enzymatic activity but bind to ssDNA cooperatively and non-specifically[16]. The stimulatory effects of SSB protein on RecA protein activity are manifest both pre- and post synaptically. In the presynaptic phase, SSB protein, by virtue of its helix-destabilization properties, removes DNA secondary structure that hinders complete presynaptic complex formation. In the postsynaptic phase (i.e. after joint-molecule formation), SSB protein binds to the displaced ssDNA, blocking reversal of DNA strand exchange[65,66]. The inhibitory effect of SSB protein is exhibited when it is allowed to bind to ssDNA prior to RecA protein. As SSB protein is a competitor for binding, it must be displaced by RecA protein. Because the binding of SSB protein to ssDNA is kinetically faster than that of RecA protein, initially more SSB-protein–ssDNA complex than RecA presynaptic complex forms; however, with time, RecA protein can displace the SSB protein. This rate-limiting displacement is avoided in the coupled process that is coordinated by RecBCD enzyme and χ: the χ-activated RecBCD enzyme ensures that RecA protein binds to ssDNA prior to SSB (Ref. 42).

E. coli also possesses proteins that function to promote the exchange of SSB for RecA protein; this is a function of the RecO(R) proteins[26,27]. Details of RecF, RecO and RecR protein action are not completely clear, but several important features have already been revealed. Biochemical analyses demonstrate a complicated mixture of pairwise interactions, as well as putative heterotrimer formation in the presence of dsDNA. RecF protein binds ATP and DNA (Refs 67,68), and has a weak ATPase activity that is stimulated by RecR protein[69]. RecF and RecR proteins block extension of a RecA filament from a ssDNA gap into the adjoining dsDNA (Ref. 70). RecO is a DNA-binding protein that has ssDNA annealing and weak D-loop formation activities[71,72]. RecO, either by itself or with RecR or RecF protein, or both, also stimulates the homologous pairing activity of RecA protein under conditions whereby an SSB–ssDNA complex is allowed to assemble prior to introduction of the RecA protein[26]. This stimulation results from an interaction of RecO protein with SSB protein that facilitates the displacement of SSB protein from ssDNA and its replacement by RecA protein[27], and from a stabilization of the RecA nucleoprotein filament against dissociation[73]. Thus, it is most interesting that both RecBCD-dependent and RecF-dependent recombination have specific, but different, mechanisms to deal with the potential competitive effects of SSB binding: χ-activated RecBCD enzyme loads RecA directly onto ssDNA, whereas the RecO complex displaces (or replaces) SSB protein in favor of RecA protein.

DNA heteroduplex extension: RuvAB and RecG proteins

The RuvAB complex is responsible for the branch migration phase of recombination, after the Holliday junction has formed[28,29]. RuvA protein targets the complex to the Holliday junction, enabling the RuvB protein to assemble as a hexamer around two opposite arms of the Holliday junction. The DNA is then 'pumped out' of the RuvAB complex by translocation in opposite directions to drive branch migration. The RecG protein possesses a similar activity, but it acts in the reverse direction to dissociate junctions[74]. RecG protein also disrupts RNA–DNA

hybrids[75] and loss of RecG protein activity enhances RDR (specifically cSDR)[76] supporting the idea that R-loops are also involved in RDR.

Holliday junction resolution: RuvC protein

RuvC protein is the endonuclease that specifically cleaves the Holliday junction[77]. It acts in conjunction with the Holliday-junction migration proteins, the RuvAB complex, to translocate the preferred cleavage sequences past the RuvC protein[29]. The RuvABC enzyme system displays a remarkable specificity for Holliday junctions, and the action of all three proteins is coordinated[78].

Although the RuvC protein acts 'late' in the recombination process to cleave the Holliday junction, it acts both late and early in RDR (Ref. 79). As will be elaborated later in this issue (see article by B. Michel in this issue of *TiBS*), RuvC nuclease acts on stalled replication forks that have regressed to form a Holliday junction. This cleavage is yet another way to create a DSB that can serve as an entry site for RecBCD enzyme, which then proceeds to produce the ssDNA necessary for RecA-protein-dependent D-loop formation. In this capacity, RuvC is acting as the 'initiator' of a DSB at the site of a stalled replication fork.

Recombination-dependent replication

Either a D-loop or an R-loop could serve as a potential primer for DNA replication. In particular, because the 3′ end of ssDNA is more invasive than the 5′ end in RecA-protein-mediated DNA strand invasion, a seemingly ideal substrate, the D-loop, is created for DNA replication. However, assembly of the DNA polymerase III holoenzyme complex is not a simple process. The relatively recent discovery that PriA protein (and its associated proteins) is required to reinitiate the replisome provides one of the last pieces to this puzzle (see article by K. Marians in this issue of *TiBS*). The PriA protein binds to the D-loop[80], initiating the orderly scheme of DNA polymerase III holoenzyme recruitment to reconstitute bidirectional DNA replication.

The studies that revealed the function of PriA protein in RDR also yielded another unexpected dividend: it was discovered that *priA* mutations drastically reduced the recombination-dependent integration of conjugal DNA or P1 phage DNA into the chromosome[15]. This finding argued that extensive DNA replication, presumably involving DNA polymerase III, was also important for homologous recombination. In fact, this finding supported a model for conjugal recombination that involved joint molecule formation at both DSB ends of the linear conjugal (or phage) DNA, followed by extensive replication to produce an 'integrated' piece of conjugal DNA within the chromosome[81]. This 'recombinant' was produced by replication of a recombination intermediate, illustrating that this process is replication-dependent recombination[12]. Thus, the processes of replication and recombination are very much intertwined.

A speculative view of recombination and RDR

If recombination is so important for replication of the chromosome under 'normal' physiological conditions (i.e. in the absence of exogenous DNA-damaging agents), then why are the *rec* and *pri* genes not essential for bacterial growth? And if the *rec* genes are not essential, why does every free-living organism (as well as bacteriophage T4) examined to date contain a recombination–DSBR system? One obvious answer is that there is much more DNA damage in the real world than in the laboratory situation – so much more so that, outside of the laboratory, damage from both exogenous agents and the normal product of intermediary metabolism is sufficient to reveal the essential nature of these proteins. Another argument is that recombination is needed to generate genetic diversity, and yet another is that recombination is non-essential because alternative pathways provide the needed functions. However, it is misleading to consider a process non-essential when 50–70% of cells are nonviable. Thus, a more accurate statement of the experimental findings is that recombination is an essential process, but only 50–70% of the cells encounter a situation that requires recombination function. Another perspective on this issue emerges when one considers the evolutionary consequences of recombination-proficient cells growing competitively with recombination-deficient cells. The fact that more than half the cells of a recombination-deficient (e.g. in a *recA*$^-$ strain) culture are nonviable confers a great selective advantage to the recombination-proficient cells, even after only 100 cell divisions (1×10^{30}), and a huge advantage on an evolutionary time scale. Thus, from an evolutionary and microbial population perspective, recombination is essential, and selective pressure will ensure that all organisms possess a recombination system. Therefore, the ubiquity of RecA-like proteins (and certain other recombination proteins) is easily explained by an obligate linkage between recombination and replication that is necessary for efficient replication of chromosomes.

Kogoma established that D-loop formation by RecA protein was an effective means of initiating DNA replication in the absence of a functioning origin of replication. Therefore, a corollary to the hypothesis presented above is that RecA-like proteins provided a simple way to initiate replication via D-loop or R-loop formation in primitive organisms. Vestiges of this mode of replication initiation can still be found in the colicin-type plasmids and in T4 late replication (see article by K. Kreuzer in this issue of *TiBS*). Although effective, this mechanism of replication is unscheduled, and therefore rather unrefined. Yet, despite the presence of an alternative, more-sophisticated, replication initiation control system, there remains an evolutionarily essential need to restart any of the replication forks that failed to progress to completion.

Finally, as mentioned above, RecA-protein-dependent pairing occurs at sites that have an intrinsic propensity for homologous pairing. This fact, with the additional knowledge that this preference is common to RecA-like proteins (E.M. Seitz and S.C.K., unpublished), is readily rationalized if one accepts the hypothesis that a major evolutionary function of RecA-like proteins, which persists to this day, is to ensure that disrupted replication is completed via an RDR-like mechanism in all organisms. There is now growing experimental evidence (especially in yeast) that recombination can offer a similar function in eukaryotes as well[82] (see articles by H. Flores-Rozas and R. Kolodner, and by A. Kass-Eisler and C. Greider in this issue of *TiBS*).

So, why do the prokaryotes require χ sites in these processes? By degrading the linear chromosomes of phages, the RecBCD enzyme protects the bacterium from phage infection. Yet, the DSB arising from phage invasion is indistinguishable from that formed by detachment of a replicating chromosome arm. The only solution to this quandary is to 'tag' the *E. coli* chromosomal DNA with a unique, over-represented DNA sequence that stops the nuclease activity of RecBCD enzyme. χ serves this function and, hence, acts as a kind of fingerprint of the bacterial genome. Furthermore, from the perspective of both recombination and RDR, the consequence of the combined action of χ and RecBCD enzyme is to process a random DSB into ssDNA with a

non-random 3′ terminus, the sequence of χ (Ref. 41), which is a preferred DNA-pairing sequence[42,61]. Therefore, the end result of processing DSBs by RecBCD enzyme and χ is to focus DNA-strand invasion events at chromosomal sites, which are intrinsically more receptive for homologous pairing and DNA strand invasion. Further, it seems to be no coincidence that most χ sites, which themselves are GT-rich, are located in GT-rich recombination islands[57] that effect a higher probability of D-loop formation. Thus, it appears that the actions of RecA and RecBCD proteins have been orchestrated to act in conjunction with a favorable class of DNA sites to promote a reaction that is evolutionarily vital to the cell, namely resumption of DNA replication.

Concluding remarks

Recent developments in each discipline have illustrated that the fields of replication, recombination and repair intersect and overlap to a large extent, mostly because these processes all involve a common substrate, DNA. Work in phage, bacteria and yeast makes it clear that efficient DNA replication under normal growth conditions requires homologous recombination. This process is defined as recombination-dependent replication (Ref. 6). Subsequent discoveries showed that, at least in *E. coli*, this collaboration between genetic systems works in both directions: efficient recombination also involves extensive DNA replication. In this case, the biological process is replication-dependent recombination[12]. The same considerations are true for DNA repair: recombination has historically been associated with repair, some of which is recombination-dependent repair. The discovery of DNA polymerases that specifically repair damaged DNA (see article by M. Goodman in this issue of *TiBS*) reveals another field of overlap in the form of a specialized replication-dependent repair system, which requires the recombination protein RecA. Thus, experimental elaboration of the elegant interdependencies between DNA replication, recombination and repair continues to demonstrate that these systems represent interconnected parts of an apparatus charged with the difficult responsibility of maintaining genomic integrity.

Acknowledgements

The author is grateful to David Bear and Andrei Kuzminov for their many helpful comments, as well as the following members of his laboratory: Rick Ando, Deana Arnold, Carole Bornarth, Piero Bianco, Cynthia Haseltine, Frank Harmon, Noriko Kantake, Julie Kleiman, Alex Mazin, Jim New, Erica Seitz and Tomohiko Sugiyama. The work in the author's laboratory is supported by grants from the NIH (AI-18987 and GM-41347) and from the Human Frontier Science Program (RG-63).

References

1 Stahl, F.W. (1979) Genetic Recombination. Thinking About It in Phage and Fungi, W.H. Freeman
2 Kuzminov, A. (1996) Recombinational Repair of DNA Damage, R.G. Landes Company
3 Kornberg, A. and Baker, T.A. (1992) DNA Replication, W.H. Freeman
4 Kuzminov, A. (1995) Collapse and repair of replication forks in *Escherichia coli*. *Mol. Microbiol.* 16, 373–384
5 Skalka, A. (1974) A replicator's view of recombination (and repair). In Mechanisms in Recombination (Grell, R.F., ed.), pp. 421–432, Plenum Press
6 Asai, T. *et al.* (1994) DNA replication triggered by double-stranded breaks in *E. coli*: dependence on homologous recombination functions. *Cell* 78, 1051–1061
7 Kuzminov, A. (1995) Instability of inhibited replication forks in *E. coli*. BioEssays 17, 733–741
8 Mosig, G. (1987) The essential role of recombination in phage T4 growth. *Annu. Rev. Genet.* 21, 347–371
9 Asai, T. *et al.* (1993) Homologous recombination-dependent initiation of DNA replication from DNA damage-inducible origins in *Escherichia coli*. *EMBO J.* 12, 3287–3295
10 Kogoma, T. (1997) Stable DNA replication: interplay between DNA replication, homologous recombination, and transcription. *Microbiol. Mol. Biol. Rev.* 61, 212–238
11 Kogoma, T. and Lark, K.G. (1970) DNA replication in *Escherichia coli*: replication in absence of protein synthesis after replication inhibition. *J. Mol. Biol.* 52, 143–164
12 Kogoma, T. (1996) Recombination by replication. *Cell* 85, 625–627
13 Nurse, P. *et al.* (1991) Inactivation of the *Escherichia coli* PriA DNA replication protein induces the SOS response. *J. Bacteriol.* 173, 6686–6693
14 Masai, H. *et al.* (1994) *Escherichia coli* PriA protein is essential for inducible and constitutive stable DNA replication. *EMBO J.* 13, 5338–5345
15 Kogoma, T. *et al.* (1996) The DNA replication priming protein, PriA, is required for homologous recombination and double-strand break repair. *J. Bacteriol.* 178, 1258–1264
16 Kowalczykowski, S.C. *et al.* (1994) Biochemistry of homologous recombination in *Escherichia coli*. *Microbiol. Rev.* 58, 401–465
17 Kowalczykowski, S.C. and Eggleston, A.K. (1994) Homologous pairing and DNA strand-exchange proteins. *Annu. Rev. Biochem.* 63, 991–1043
18 Szostak, J.W. *et al.* (1983) The double-strand break repair model for recombination. *Cell* 33, 25–35
19 Roca, A.I. and Cox, M.M. (1997) RecA protein: structure, function, and role in recombinational DNA repair. *Prog. Nucleic Acid Res. Mol. Biol.* 56, 129–223
20 Bianco, P.R. *et al.* (1998) DNA strand exchange proteins: A biochemical and physical comparison. *Front Biosci.* 3, 570–603
21 Bianco, P.R. and Kowalczykowski, S.C. (1999) RecA protein. In *Encyclopedia of Life Sciences*, Nature Publishing Group, London, http://www.els.net
22 Mazin, A.V. and Kowalczykowski, S.C. (1999) A novel property of the RecA nucleoprotein filament: activation of double-stranded DNA for strand exchange in trans. *Genes Dev.* 13, 2005–2016
23 Zaitsev, E.N. and Kowalczykowski, S.C. A novel pairing process promoted by *Escherichia coli* RecA protein: Inverse DNA and RNA strand exchange. *Genes Dev.* (in press)
24 Arnold, D.A. and Kowalczykowski, S.C. (1999) RecBCD helicase/nuclease. In *Encyclopedia of Life Sciences*, Nature Publishing Group, London, http://www.els.net
25 Smith, G.R. (1989) Homologous recombination in *E. coli*: multiple pathways for multiple reasons. *Cell* 58, 807–809
26 Umezu, K. *et al.* (1993) Biochemical interaction of the *Escherichia coli* RecF, RecO, and RecR proteins with RecA protein and single-stranded DNA binding protein. *Proc. Natl. Acad. Sci. U. S. A.* 90, 3875–3879
27 Umezu, K. and Kolodner, R.D. (1994) Protein interactions in genetic recombination in *Escherichia coli*. Interactions involving RecO and RecR overcome the inhibition of RecA by single-stranded DNA-binding protein. *J. Biol. Chem.* 269, 30005–30013
28 West, S.C. (1996) DNA helicases: new breeds of translocating motors and molecular pumps. *Cell* 86, 177–180
29 West, S.C. (1996) The RuvABC proteins and Holliday junction processing in *Escherichia coli*. *J. Bacteriol.* 178, 1237–1241
30 Smith, G.R. (1990) RecBCD enzyme. In *Nucleic Acids and Molecular Biology* (Vol. 4) (Eckstein, F. and Lilley, D.M.J., eds.), pp. 78–98, Springer-Verlag
31 Wang, T-C.V. and Smith, K.C. (1983) Mechanisms for *recF*-dependent and *recB*-dependent pathways of postreplication repair in UV-irradiated *Escherichia coli uvrB*. *J. Bacteriol.* 156, 1093–1098
32 Magee, T.R. and Kogoma, T. (1990) Requirement of RecBC enzyme and an elevated level of activated RecA for induced stable DNA replication in *Escherichia coli*. *J. Bacteriol.* 172, 1834–1839
33 Harris, R.S. *et al.* (1994) Recombination in adaptive mutation. *Science* 264, 258–260
34 Roman, L.J. and Kowalczykowski, S.C. (1989) Characterization of the helicase activity of the *Escherichia coli* RecBCD enzyme using a novel helicase assay. *Biochemistry* 28, 2863–2873
35 Roman, L.J. and Kowalczykowski, S.C. (1989) Characterization of the adenosinetriphosphatase activity of the *Escherichia coli* RecBCD enzyme: Relationship of ATP hydrolysis to the unwinding of duplex DNA. *Biochemistry* 28, 2873–2881
36 Roman, L.J. *et al.* (1992) Processivity of the DNA helicase activity of *Escherichia coli* recBCD enzyme. *J. Biol. Chem.* 267, 4207–4214
37 Taylor, A. and Smith, G.R. (1980) Unwinding and rewinding of DNA by the recBC enzyme. *Cell* 22, 447–457
38 Dixon, D.A. and Kowalczykowski, S.C. (1991) Homologous pairing *in vitro* stimulated by the recombination hotspot, Chi. *Cell* 66, 361–371
39 Dixon, D.A. and Kowalczykowski, S.C. (1993) The recombination hotspot χ is a regulatory sequence that acts by attenuating the nuclease activity of the *E. coli* RecBCD enzyme. *Cell* 73, 87–96
40 Anderson, D.G. and Kowalczykowski, S.C. (1997) The recombination hot spot χ is a regulatory element that switches the polarity of DNA degradation by the RecBCD enzyme. *Genes Dev.* 11, 571–581
41 Taylor, A.F. and Smith, G.R. (1995) Strand specificity of nicking of DNA at Chi sites by RecBCD enzyme. Modulation by ATP and magnesium levels. *J. Biol. Chem.* 270, 24459–24467
42 Anderson, D.G. and Kowalczykowski, S.C. (1997) The translocating RecBCD enzyme stimulates recombination by directing RecA protein onto ssDNA in a χ-regulated manner. *Cell* 90, 77–86
43 Arnold, D.A. and Kowalczykowski, S.C. Facilitated loading of RecA protein by RecBCD enzyme is essential to recombination. *J. Biol. Chem.* (in press)
44 Dixon, D.A. *et al.* (1994) Reversible inactivation of the *Escherichia coli* RecBCD enzyme by the recombination hotspot χ *in vitro*: evidence for functional inactivation or loss of the RecD subunit. *Proc. Natl. Acad. Sci. U. S. A.* 91, 2980–2984
45 Korangy, F. and Julin, D.A. (1993) Kinetics and processivity of ATP hydrolysis and DNA unwinding by the RecBC enzyme from *Escherichia coli*. *Biochemistry* 32, 4873–4880
46 Churchill, J.J. *et al.* (1999) The RecBC enzyme loads RecA protein onto ssDNA asymmetrically and independently of Chi, resulting in constitutive recombination activation. *Genes Dev.* 13, 901–911
47 Nakayama, H. *et al.* (1984) Isolation and genetic characterization of a thymineless death-resistant mutant of *Escherichi coli* K-12: Identification of a new mutation (*recQ1*) that blocks the recF recombination pathway. *Mol. Gen. Genet.* 195, 474–480
48 Mendonca, V.M. *et al.* (1995) DNA helicases in recombination and repair: construction of a *ΔuvrD ΔhelD ΔrecQ* mutant deficient in recombination and repair. *J. Bacteriol.* 177, 1326–1335
49 Umezu, K. *et al.* (1990) *Escherichia coli* RecQ protein is a DNA helicase. *Proc. Natl. Acad. Sci. U. S. A.* 87, 5363–5367
50 Harmon, F.G. and Kowalczykowski, S.C. (1998) RecQ helicase, in concert with RecA and SSB proteins, initiates and disrupts DNA recombination. *Genes Dev.* 12, 1134–1144
51 Lam, S.T. *et al.* (1974) Rec-mediated recombinational hot spot activity in bacteriophage lambda. II. A mutation which causes hot spot activity. *Genetics* 77, 425–433
52 Source, S. *et al.* (1998) Identification of the Chi site of *Haemophilus influenzae* as several sequences related to the *Escherichia coli* Chi site. *Mol. Microbiol.* 27, 1021–1029

53 el Karoui, M. *et al.* (1998) Identification of the lactococcal exonuclease/recombinase and its modulation by the putative Chi sequence. *Proc. Natl. Acad. Sci. U. S. A.* 95, 626–631
54 Chédin, F. *et al.* The *Bacillus subtilis* AddAB helicase/nuclease is regulated by its cognate Chi sequence *in vitro*. *J. Mol. Biol.* (in press)
55 Stahl, F.W. *et al.* (1990) Further tests of a recombination model in which Chi removes the RecD subunit from the RecBCD enzyme of *Escherichia coli*. *Genetics* 126, 519–533
56 Bianco, P.R. and Kowalczykowski, S.C. (1997) The recombination hotspot Chi is recognized by the translocating RecBCD enzyme as the single strand of DNA containing the sequence 5′-GCTGGTGG-3′. *Proc. Natl. Acad. Sci. U. S. A.* 94, 6706–6711
57 Tracy, R.B. *et al.* (1997) The recombination hot spot Chi is embedded within islands of preferred DNA pairing sequences in the *E. coli* genome. *Cell* 90, 205–206
58 Kuzminov, A. *et al.* (1994) Chi sites in combination with RecA protein increase the survival of linear DNA in *Escherichia coli* by inactivating exoV activity of RecBCD nuclease. *EMBO J.* 13, 2764–2776
59 Kowalczykowski, S.C. (1991) Biochemistry of genetic recombination: energetics and mechanism of DNA strand exchange. *Annu. Rev. Biophys. Biophys. Chem.* 20, 539–575
60 von Hippel, P.H. and Berg, O.G. (1989) Facilitated target location in biological systems. *J. Biol. Chem.* 264, 675–678
61 Tracy, R.B. and Kowalczykowski, S.C. (1996) *In vitro* selection of preferred DNA pairing sequences by the *Escherichia coli* RecA protein. *Genes Dev.* 10, 1890–1903
62 Beattie, K.L. *et al.* (1977) Uptake of homologous single-stranded fragments by superhelical DNA: II. Characterization of the reaction. *J. Mol. Biol.* 116, 783–803
63 Kasahara, M. *et al.* (2000) RecA protein-dependent R-loop formation *in vitro*. *Genes Dev.* 14, 360–365
64 Kowalczykowski, S.C. *et al.* (1987) Effects of the *Escherichia coli* SSB protein on the binding of *Escherichia coli* RecA protein to single-stranded DNA: Demonstration of competitive binding and the lack of a specific protein–protein interaction. *J. Mol. Biol.* 193, 81–95
65 Lavery, P.E. and Kowalczykowski, S.C. (1992) A postsynaptic role for single-stranded DNA-binding protein in recA protein-promoted DNA strand exchange. *J. Biol. Chem.* 267, 9315–9320
66 Mazin, A.V. and Kowalczykowski, S.C. (1998) The function of the secondary DNA-binding site of RecA protein during DNA strand exchange. *EMBO J.* 17, 1161–1168
67 Griffin, T.J. and Kolodner, R.D. (1990) Purification and preliminary characterization of the *Escherichia coli* K-12 recF protein. *J. Bacteriol.* 172, 6291–6299
68 Madiraju, M.V.V.S. and Clark, A.J. (1992) Evidence for ATP binding and double-stranded DNA binding by *Escherichia coli* RecF protein. *J. Bacteriol.* 174, 7705–7710
69 Webb, B.L. *et al.* (1995) An interaction between the *Escherichia coli* RecF and RecR proteins dependent on ATP and double-stranded DNA. *J. Biol. Chem.* 270, 31397–31404
70 Webb, B.L. *et al.* (1997) Recombinational DNA repair: the RecF and RecR proteins limit the extension of RecA filaments beyond single-strand DNA gaps. *Cell* 91, 347–356
71 Luisi-DeLuca, C. and Kolodner, R.D. (1994) Purification and characterization of the *Escherichia coli* RecO protein. Renaturation of complementary single-stranded DNA molecules catalyzed by the RecO protein. *J. Mol. Biol.* 236, 124–138
72 Luisi-DeLuca, C. (1995) Homologous pairing of single-stranded DNA and superhelical double-stranded DNA catalyzed by RecO protein from *Escherichia coli*. *J. Bacteriol.* 177, 566–572
73 Shan, Q. *et al.* (1997) RecA protein filaments: end-dependent dissociation from ssDNA and stabilization by RecO and RecR proteins. *J. Mol. Biol.* 265, 519–540
74 Whitby, M.C. *et al.* (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
75 Vincent, S.D. *et al.* (1996) The RecG branch migration protein of *Escherichia coli* dissociates R-loops. *J. Mol. Biol.* 264, 713–721
76 Hong, X. *et al.* (1995) *Escherichia coli* RecG and RecA proteins in R-loop formation. *EMBO J.* 14, 2385–2392
77 Connolly, B. *et al.* (1991) Resolution of Holliday junctions *in vitro* requires the *Escherichia coli ruvC* gene product. *Proc. Natl. Acad. Sci. U. S. A.* 88, 6063–6067
78 Eggleston, A.K. *et al.* (1997) *In vitro* reconstitution of the late steps of genetic recombination in *E. coli*. *Cell* 89, 607–617
79 Seigneur, M. *et al.* (1998) RuvAB acts at arrested replication forks. *Cell* 95, 419–430
80 McGlynn, P. *et al.* (1997) The DNA replication protein PriA and the recombination protein RecG bind D-loops. *J. Mol. Biol.* 270, 212–221
81 Smith, G.R. (1991) Conjugational recombination in *E. coli*: myths and mechanisms. *Cell* 64, 19–27
82 Paques, F. and Haber, J.E. (1999) Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* 63, 349–404

Recombination-dependent DNA replication in phage T4

Kenneth N. Kreuzer

Studies in the 1960s implied that bacteriophage T4 tightly couples DNA replication to genetic recombination. This contradicted the prevailing wisdom of the time, which staunchly supported recombination as a simple cut-and-paste process. More-recent investigations have shown how recombination triggers DNA synthesis and why the coupling of these two processes is important. Results from T4 were instrumental in our understanding of many important replication and recombination proteins, including the newly recognized replication/recombination mediator proteins. Recombination-dependent DNA replication is crucial to the T4 life cycle as it is the major mode of DNA replication and is also central to the repair of DNA breaks and other damage.

THE INITIATION OF extensive DNA replication by recombination was first recognized in the bacteriophage T4 system. Mutations that inactivate phage-encoded recombination proteins were found to cause the 'DNA-arrest' phenotype, a profound defect in which phage DNA replication begins normally but then quickly ceases[1]. Mosig[2] explained this and other results by proposing that most phage DNA replication initiates at the 3′ ends of D-loops created by strand invasion (Fig. 1). Such D-loops form when a single-stranded (ss) 3′ end invades homologous duplex DNA, displacing a single strand of the same polarity from the duplex. For about a decade after this proposal, most researchers in the field dismissed this as nothing more than a bizarre complexity of a baroque phage life cycle. However, as described elsewhere in this issue of *TiBS*, recombination-dependent DNA replication (RDR) has now taken center stage in the maintenance of bacterial and eukaryotic genomes.

Results from the T4 system provide a detailed view of the molecular mechanism of RDR. Early genetic results demonstrated that T4 recombination occurs preferentially at the ends of the infecting phage chromosomes in a process that is linked to DNA replication[2,3]. The importance of DNA ends was confirmed and extended by *in vivo* studies demonstrating that artificially induced double-strand breaks (DSBs) trigger RDR (Refs 4–6). Also, as alluded to above, *in vivo* results implicated the phage-encoded recombination proteins UvsX, UvsY, gp32, gp46/47 and gp59 in RDR (Table 1). With the exception of gp46/47, the key function of each of these proteins in RDR has been elucidated in a series of biochemical studies with purified proteins. This article will mainly focus on the importance of RDR for phage DNA replication and repair, and on key molecular aspects of RDR that have emerged from the T4 system.

The importance of RDR for T4 chromosome ends

The infecting chromosome of phage T4 is a linear duplex DNA molecule. As with all other linear DNA molecules, this

K.N. Kreuzer is at the Dept of Microbiology, Duke University Medical Center, Durham, NC 27710, USA.
Email: Kenneth.Kreuzer@Duke.edu

 PII: S0968-0004(00)01559-0