The Bacterial Chromosome
Edited by N. Patrick Higgins

Chapter 21

Homologous Recombination by the RecBCD and RecF Pathways

MARIA SPIES AND STEPHEN C. KOWALCZYKOWSKI

RECOMBINATIONAL REPAIR OF DNA DAMAGE

Homologous, or general, recombination is a crucial biological process that involves the paring and transfer of strands between DNA molecules that share a region of significant sequence homology. After its discovery in 1946 by Lederberg and Tatum (48), homologous recombination in bacteria was associated with the sexual process of conjugation and was viewed as an evolutionary mechanism both for shuffling the genome and for spreading favorable alleles. However, more recently, a more immediate function of homologous recombination has been recognized: namely, it is a mechanism for the maintenance of chromosomal integrity that acts to repair DNA lesions, both double-strand DNA breaks and single-strand DNA gaps, generated during the course of DNA replication. The intimate connection between the processes of replication and recombination was initially appreciated in the life cycle of bacteriophage T4 (56) and then later recognized as an important determinant of viability in bacteria (39, 45). In T4 phage, recombination is linked to replication to produce a high yield of phage DNA; in *Escherichia coli*, recombination is linked to replication to permit its completion when interrupted by DNA damage, and also to initiate DNA replication in the absence of origin function. This view of recombination as an integral part of efficient chromosome duplication also reconciled the high level of inviability (up to 95%) of recombination-deficient cells (12).

GENERATION OF A DSB IN DNA

A significant fraction of DNA damage affects only one strand of the DNA duplex. Such lesions can often be repaired by one of the repair systems that are specific for a particular DNA lesion (see reference 28 for a review). These repair systems use the intact complementary strand as a template to restore the damaged DNA molecule to its original state. Occasionally both strands of the double-stranded DNA (dsDNA) can be broken opposite to each other, resulting in a double-strand break (DSB). DSBs can be produced, for example, as a direct consequence of ionizing radiation (Fig. 1A). However, the bulk of the DSBs in bacteria are generated indirectly as the result of DNA replication through an unrepaired break in just a single strand of DNA (Fig. 1B). Replication of DNA containing a single-strand nick or a gap in the leading strand results in the dissociation of the DNA polymerase holoenzyme complex and the generation of one blunt DSB (Fig. 1B), whereas replication of DNA with a nick in the lagging strand produces a DSB with a 3′-terminated single-stranded DNA (ssDNA) tail.

A DSB can also be produced when the DNA replication process is halted by anything that might block the progress of the replisome. Upon dissociation of the replisome, the stalled replication fork can regress to produce a Holliday junction. Such structure contains both a dsDNA end and an intermediate of recombination and, hence, attracts recombination machinery in a manner similar to that of the simple DSB. In addition, the Holliday junction can be cleaved to provide yet another path for DSB formation (Fig. 2). Left unrepaired, DSBs are lethal, and *E. coli* can repair only a few DSBs per chromosome without dying (43). Thus, DNA replication is a major source of endogenous DSBs that, in turn, are repaired by homologous recombination. This relationship between DNA replication and recombinational repair of DSBs is summarized in Fig. 1.

Recombinational repair, however, requires a homologous DNA molecule to be used as a template from which to restore, by DNA synthesis, the genetic

Maria Spies and Stephen C. Kowalczykowski • Sections of Microbiology and of Molecular and Cellular Biology, Center for Genetics and Development, University of California, Davis, CA 95616-8665.

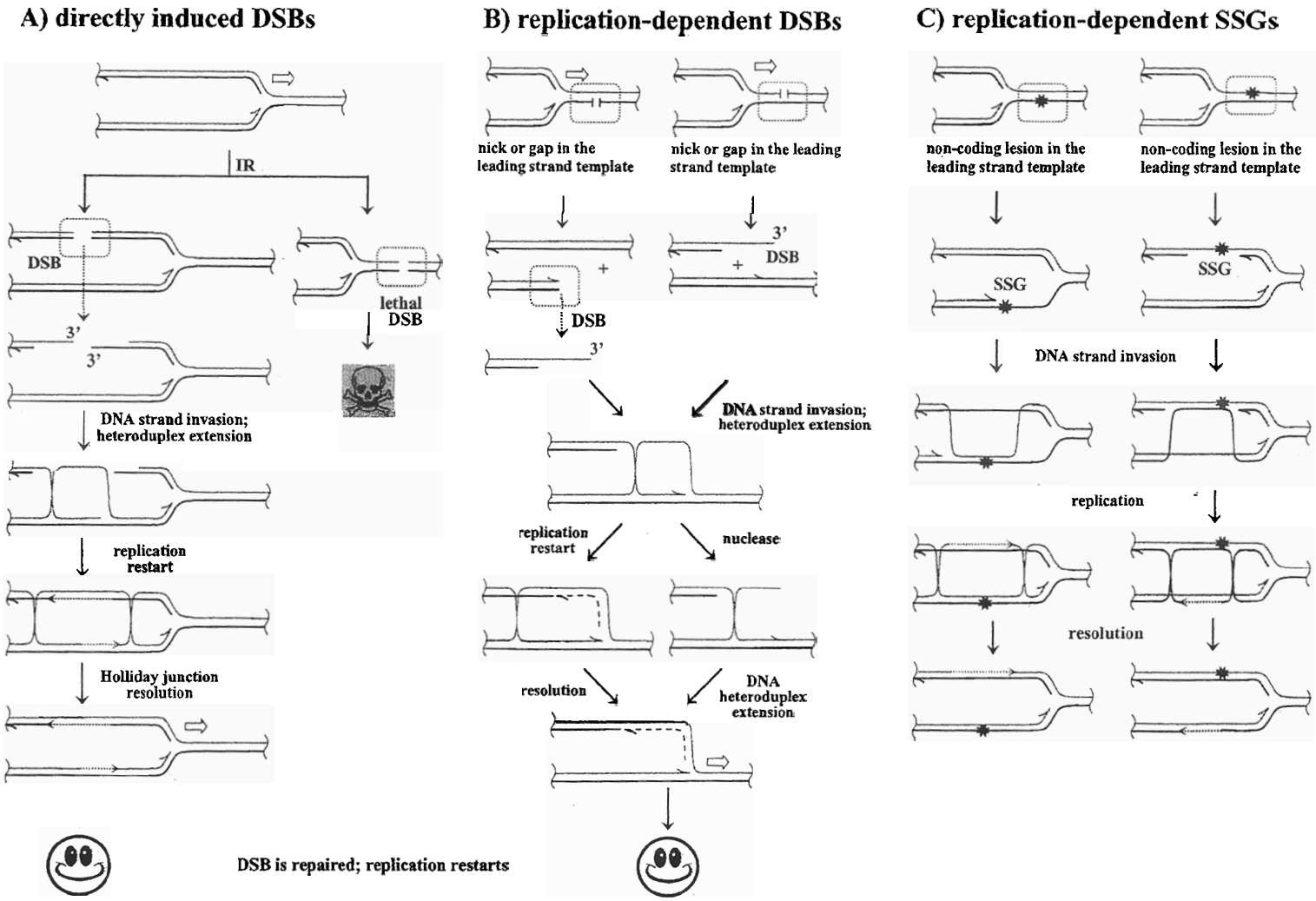

Figure 1. Recombinational repair of DNA damage. The DNA strands that are used as templates for the leading strand synthesis are shown in black, and the strands used as templates for the lagging strand synthesis are shown in gray. Arrows indicate the direction of DNA synthesis. (A) Recombinational repair of directly induced DSBs. A DSB, which occurs in newly synthesized DNA, can be repaired by completion of the following steps. First, the DSB is processed to produce 3′-terminated ssDNA. Then, one of the ssDNA tails can invade the homologous dsDNA daughter, displacing one of the resident strands to form a D-loop. This structure can be used as a template for DNA synthesis, ultimately resulting in the formation of a Holliday junction. Upon resolution of the Holliday junction, the replication fork is restored to its original form. When a DSB occurs in a part of the chromosome that is not yet replicated, there is no homologous DNA to serve as a template, and such a DSB can be lethal. (B) Recombinational repair of replication-dependent DSBs. DSBs can be produced by

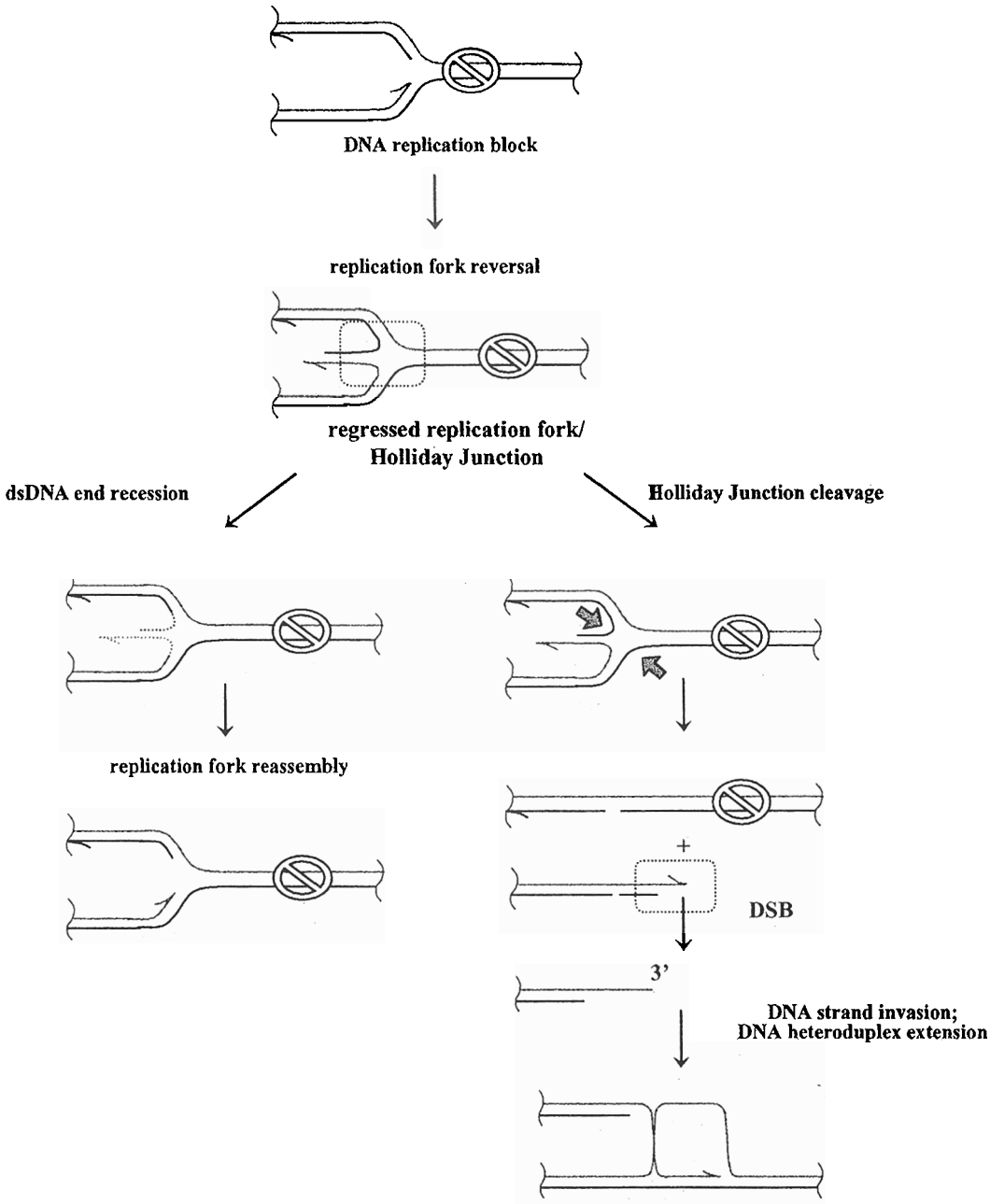

Figure 2. DSBs can be produced as a result of Holliday junction cleavage. Reversal of a stalled DNA replication fork results in the formation of a regressed replication fork, which is a four-way (Holliday) junction that contains a dsDNA end. There are two potential fates for this regressed replication fork: it can be degraded by the recombination machinery to produce a three-way junction that resembles a replication fork, or the Holliday junction can be cleaved to produce a DSB that is repaired by a DSB repair pathway.

replication through a single-strand DNA break. The source of the replication-dependent DSBs is a nick or an ssDNA gap in one of the strands of the replicated DNA molecule. Replication through the strand discontinuity results in the formation of one intact and one broken DNA molecule. The end of the broken chromosome is processed to form a 3′-terminated ssDNA tail, which invades the intact homologous DNA to form a D-loop, which can then be used to restore a normal replication fork. (C) Recombinational repair of replication-dependent SSGs. SSGs can be produced when synthesis of only one DNA strand is halted by an encounter of a noncoding lesion in that DNA strand. After DNA strand exchange of the ssDNA in the gap with a strand in the intact daughter homologue, the displaced DNA strand can be used as a template for DNA synthesis, resulting in the restoration of the replication fork. Upon completion of replication, one of the DNA molecules will still contain the original DNA damage. If this damage is not repaired by the appropriate repair system, then an SSG will be formed again by the next round of DNA replication.

content of the damaged DNA molecule. These homologous DNA sequences are generated by DNA replication, and, therefore, the meridiploid character of bacterial chromosomes provides the necessary templates for recombinational repair.

GENERATION OF A SINGLE-STRAND GAP IN DNA

When synthesis of a DNA strand is blocked by a noncoding lesion (for example, an abasic site or an intrastrand cross-link, such as a thymine dimer), continued replication of the flawless strand beyond the lesion produces a single-strand DNA gap (SSG) (Fig. 1C) (42, 54). SSGs are also known as daughter-strand gaps, since they appear on one of the newly synthesized daughter strands during semiconservative DNA replication.

The interstrand DNA cross-link is a special class of chemical damage to DNA, since it blocks DNA replication completely by preventing DNA strand separation (Fig. 3) (28). Repair of this special class of DNA damage is absolutely dependent on homologous recombination (65). Even a single cross-link is lethal for recombination-deficient cells, while wild-type *E. coli* cells can tolerate up to 70 cross-links per chromosome (65). DNA cross-links are repaired through an incisional-recombinational mechanism. An incision is made at both sides of the cross-link on one DNA strand, and displacement of the incised oligonucleotide results in an SSG that can be repaired through an SSG repair pathway. Subsequent incision on the second strand releases the cross-linked strands, producing a second SSG that is repaired by a second round of recombinational repair.

For all SSGs, if the ssDNA in the gap is cleaved before the completion of SSG repair, then the lesion

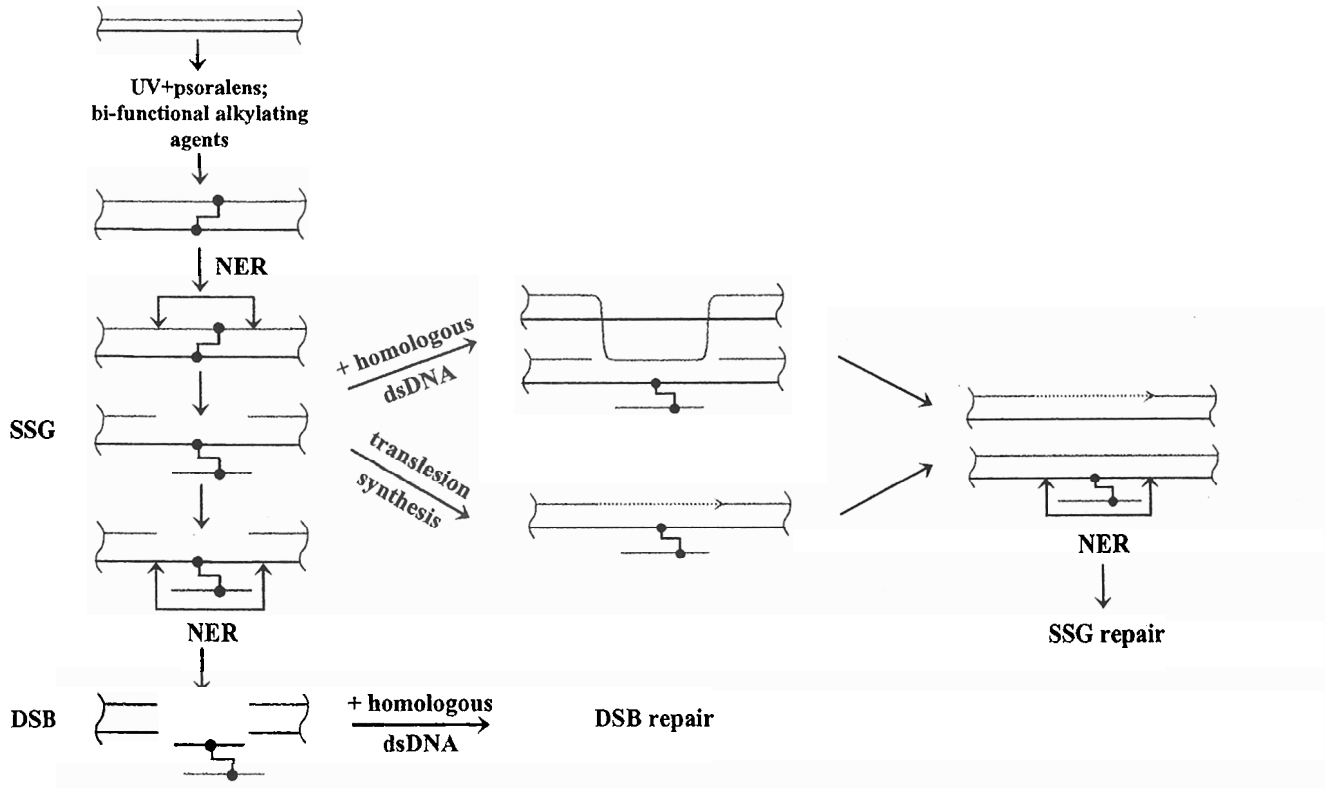

Figure 3. Interstrand cross-links are converted into DSBs and SSGs. Schematic representation for the incision-recombination mechanism of interstrand DNA cross-link repair. The repair of an interstrand cross-link depends on both the nucleotide excision repair (NER) and recombination machineries. The NER enzymes make an incision on either side of the lesion on one DNA strand and also displace the cross-link-containing oligonucleotide to produce an SSG; this SSG can be repaired by an SSG repair pathway. However, the incised oligonucleotide remains cross-linked to the second DNA strand. If this lesion is recognized by NER machinery prior to the completion of SSG repair of the top strand, then the subsequent incisions on the second strand convert the interstrand cross-link into a DSB, which is then repaired by a DSB repair pathway.

is converted into a DSB. Thus, some fraction of SSGs are converted into DSBs and are repaired through the DSB repair pathway.

CONJUGATION, TRANSDUCTION, AND TRANSFORMATION CREATE DSBs AND SSGs

DSBs arise not only as a consequence of DNA damage; in fact, DSBs are formed as a natural step in several normal cellular processes. The molecular feature common to each of these processes is the cellular acquisition of a linear segment of dsDNA. The end of this linear DNA molecule is seen as a DSB, and the recombinational repair of the DSB is initiated. Bacteria can acquire linear DNA by any of three different routes. First, during conjugation, a copy of a chromosome is transferred from one bacterium, which possesses a fertility factor (the F′ plasmid), to another. Genetic studies of this process led to isolation of the first recombination-deficient mutants (21). Transformation is a second route by which some bacteria take up a segment of DNA from the environment; unrelated in process, but not in the form of the DNA involved, artificial transformation is widely used as a laboratory technique to introduce foreign dsDNA into cells. Finally, infection by bacteriophage also results in the introduction of linear DNA into bacteria. Note, however, that in addition to DSBs, the processes of conjugation and natural transformation also produce SSGs due to incomplete replication of the DNA intermediates that form during these processes.

HOMOLOGOUS PAIRING AND DNA STRAND EXCHANGE MEDIATED BY RecA PROTEIN

A step common to all pathways of recombinational repair discussed below is the homologous pairing of DNA (Fig. 1). In bacteria, the ubiquitous RecA protein catalyzes an invasion of ssDNA into homologous duplex DNA and the exchange of DNA strands. Regardless of whether the DNA lesion is a DSB or an SSG, pathway-specific processing of the break produces an extensive region of ssDNA that serves as the substrate for assembly of the RecA nucleoprotein filament. The process of finding DNA sequence homology and exchanging DNA strands occurs in three defined stages: (i) presynapsis, during which the RecA nucleoprotein is assembled; (ii) synapsis, during which the homology search and exchange of DNA strands occur; and (iii) postsynapsis, during which branch migration can occur. Afterward, resolution of the resulting recombination intermediate produces a recombinant molecule.

In *E. coli*, *recA* null mutations reduce conjugational recombination by 100,000-fold (21). The active form of RecA protein is a nucleoprotein filament formed by the cooperative binding of RecA protein to the ssDNA tails of the processed DSB, or to the ssDNA in the SSG (see reference 6 for a review). Thus, ssDNA is an essential, although transient, intermediate in the process of homologous recombination. Most of the SSGs, whose average length is minimally 200 nucleotides (78) (but can be as large as 800 nucleotides [37], or half the average size of an Okazaki fragment [66]), are sufficiently large for RecA nucleoprotein filament assembly; however, DSBs are not the normal direct targets of RecA protein binding. Therefore, DSBs and short SSGs are processed to produce longer regions of ssDNA. This processing requires either a nuclease, a helicase, or both (see below).

However, production of ssDNA is not sufficient for RecA nucleoprotein filament assembly in vivo. Within the cell, ssDNA rapidly forms a complex with the ssDNA-binding (SSB) protein. SSB protein binding has the beneficial consequences of protecting ssDNA from degradation by nucleases and of disrupting inhibitory DNA secondary structures. Unfortunately, SSB protein blocks assembly of the RecA nucleoprotein filament by competing directly with RecA protein for ssDNA binding. Thus, the SSB protein-ssDNA complex is a kinetic barrier for the assembly of a RecA-ssDNA nucleoprotein filament, and its formation is inhibitory for the subsequent steps of homologous recombination. However, to counteract this inhibitory effect of SSB protein, there is a class of the recombination/replication mediator proteins whose function is to facilitate assembly of RecA protein (and its homologues in other organisms) on ssDNA, thereby alleviating the kinetic barrier imposed by SSB protein (the role of replication mediator proteins is reviewed in reference 5). Thus, in addition to the production of a single-stranded region within dsDNA, recombination requires the "loading" of RecA protein onto this ssDNA.

In wild-type *E. coli*, the processing of a broken DNA molecule, and the subsequent delivery of RecA protein to this ssDNA, occurs by either of two pathways: RecBCD or RecF. The pathway names reflect critical and unique enzymes acting in each. The RecBCD pathway is used primarily to initiate recombination at a DSB, whereas the RecF pathway is used for recombinational repair at SSGs. The concept

of recombination pathways was initially postulated by Clark (20). In both pathways, the Holliday junctions that result are resolved by the RuvABC enzyme complex (see reference 46 for a review) into the recombinant progeny.

DSB REPAIR BY RECOMBINATION: THE RecBCD PATHWAY

The RecBCD pathway comprises the RecBCD, SSB, RecA, and RuvABC proteins; in addition, a specific DNA locus called χ (Chi [crossover hot spot instigator], 5′-GCTGGTGG-3′) is required in the dsDNA that is broken.

In wild-type *E. coli*, RecBCD enzyme is required for approximately 99% of the recombination events associated with conjugation and transduction, i.e., the processes that involve linear DNA and, hence, a DSB. Genetic studies revealed that the products of *recB* and *recC* genes are necessary for the repair of DSBs and for conjugational recombination. Deletion of either *recB* or *recC* genes reduces the levels of sexual recombination 100- to 1,000-fold and increases sensitivity to DNA-damaging agents, such as UV irradiation and mitomycin C (26, 36, 84). This effect, however, is significantly smaller then the 100,000-fold decrease in homologous recombination in the *recA* mutant cells. In contrast, cells deleted for *recD* are not only proficient in homologous recombination and DSB repair, but they also catalyze homologous recombination at high rates (1).

The RecBCD enzyme is a heterotrimer consisting of three nonidentical polypeptides, RecB (134 kDa), RecC (129 kDa), and RecD (67 kDa) (1, 58). The three subunits compose a complex, multifunctional enzyme that possesses a number of seemingly disparate catalytic activities, including DNA-dependent ATPase, DNA helicase, ssDNA endo- and exonuclease, and dsDNA exonuclease. In vivo, these activities enable RecBCD enzyme to carry out a highly coordinated set of biochemical reactions resulting in conversion of the broken dsDNA into the active species in homologous recombination: the RecA nucleoprotein filament assembled on the ssDNA containing χ.

Among DNA helicases, an unusual feature of the RecBCD enzyme is its ability to initiate unwinding at a blunt or nearly blunt dsDNA end. RecBCD enzyme binds to blunt DNA ends with a very high affinity ($\sim$1 nM), forming an initiation complex (30) that has a footprint of about 20 or 21 nucleotides on the 5′-terminated strand and 16 to 17 nucleotides on the 3′-terminated strand.

RecB protein has a modular organization. The N-terminal domain of the RecB subunit contains motifs characteristic for the superfamily 1 (SF1) DNA helicases. Moreover, the purified RecB protein is an ssDNA-dependent ATPase and a DNA helicase (10). Its behavior is related to the well-characterized Rep, UvrD, and PcrA helicases. Similar to these enzymes, RecB protein displays 3′ $\rightarrow$ 5′ helicase activity, requiring a 3′-terminated ssDNA region flanking the duplex DNA that is to be unwound. The C-terminal domain of this same subunit contains a nuclease motif resembling that of other nucleases, such as FokI and BamHI (77, 85) and λ exonuclease (2c). Besides being responsible for the complicated nuclease activity of RecBCD enzyme, the C-terminal domain of the RecB subunit has another crucial function: it harbors a site for interaction with RecA protein (19).

The amino acid sequence of RecC protein provides no clue as to its role in the holoenzyme. However, the existence of the RecC mutants that enable the holoenzyme to recognize an altered χ sequence suggests a significant role in χ recognition (3). Interaction with the RecC subunit greatly stimulates the weak nuclease activity of the RecB helicase, and increases its affinity for dsDNA ends (61). The resulting RecBC enzyme is a fast, processive helicase that can initiate homologous recombination. However, RecBC enzyme displays negligible nuclease activity. As a result, homologous recombination in the *recD* mutant cells strongly depends on the function of RecJ nuclease (51, 51a).

The RecD subunit also contains SF1 helicase motifs. The purified RecD protein is a DNA-dependent ATPase that was recently shown to be a 5′ $\rightarrow$ 3′ helicase (23) similar to the closely related TraI and Dda helicases. Like these other helicases, RecD protein unwinds substrates containing 5′-terminated ssDNA flanking the duplex DNA. Interaction with the RecD subunit further stimulates both the helicase activity and the dsDNA end-binding affinity of RecBC enzyme. Given that the RecB subunit is bound to the 3′-terminated strand, the RecD subunit is bound to the 5′-terminated strand (27), and each motor subunit moves with an opposite polarity, the resulting bipolar RecBCD enzyme translocates with each motor subunit moving in the same direction relative to the DSB. In addition, the resulting RecBCD holoenzyme now manifests its vigorous nuclease activity, implying that the RecD subunit also activates the nuclease contained within the RecB subunit (41).

The activities described above permit the following description of the mechanism of action of the RecBCD helicase/nuclease (Fig. 4). Upon binding to the DNA end (Fig. 4a), the enzyme uses the energy of ATP hydrolysis to translocate along and unwind the dsDNA molecule, consuming approximately two

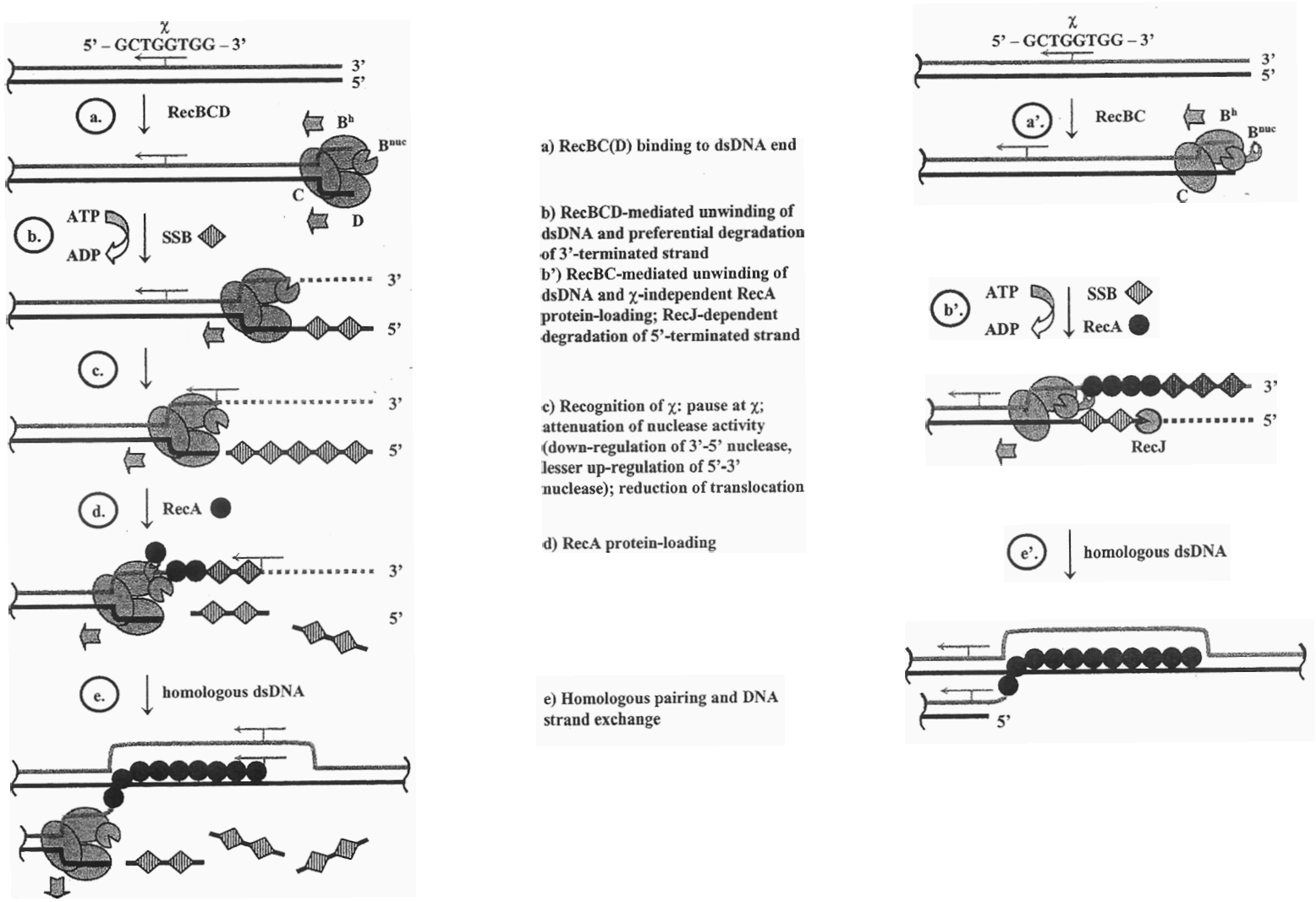

Figure 4. Initiation of homologous recombination at DSBs by the RecBCD pathway. Schematic representation of the early enzymatic steps of RecBCD pathway of recombinational repair. (a and a′) RecBCD (or RecBC) enzyme binds to the blunt or nearly blunt dsDNA end. (b) RecBCD enzyme uses the energy of ATP hydrolysis to translocate along and to unwind the dsDNA. The associated nuclease activity degrades the newly produced ssDNA. (c) Interaction with χ results in the attenuation of the RecBCD nuclease activity and production of ssDNA terminated with the χ sequence at its 3′ end. (d) The χ-modified enzyme loads RecA protein onto the χ-containing ssDNA to the exclusion of SSB protein, forming a RecA nucleoprotein filament. (e and e′) The RecA-ssDNA nucleoprotein filament invades homologous dsDNA. (b′) The RecBC enzyme (i.e., lacking the RecD subunit) behaves as a constitutively χ-modified RecBCD enzyme that can load RecA protein without the need for χ interaction. The RecBC enzyme lacks nuclease activity that is compensated for by the action of the RecJ nuclease.

molecules of ATP per base pair unwound (63) (Fig. 4b). RecBCD enzyme unwinds, on average, 30,000 bp of dsDNA per binding event (62) at a rate of approximately 1,000 to 1,300 bp/s (at 37°C). DNA unwinding by RecBCD enzyme is accompanied by endonucleolytic cleavage of the newly produced ssDNA. The nuclease activity of RecBCD enzyme is asymmetric, with digestion occurring preferentially on the 3′-terminated strand relative to the DSB (25).

THE RECOMBINATION HOT SPOT, χ

Originally, χ was identified as a *cis*-acting mutation in bacteriophage λ that allowed its more efficient growth in *E. coli* by stimulating the host's recombination system. Stimulation of recombination is approximately 10-fold (47), and it is confined to loci that are downstream of χ (70): stimulation is highest at χ and then decreases exponentially (17, 47). The χ sequence is overrepresented in *E. coli*: 1,009 χ sequences are found in the 4.6-Mb genome of the MG1655 strain (9). Furthermore, over 60% of χ sequences are oriented toward replication origin. Such an orientation would facilitate RecBCD-mediated recombination repair of DSBs created during DNA replication (11).

Biochemically, the activities of RecBCD enzyme are altered upon recognition of χ. Alteration of RecBCD enzyme activity is manifested only when the enzyme approaches χ, 5′-GCTGGTGG-3′, from its 3′ side. In the schematic depiction (Fig. 4), χ, which is the sequence in the "top strand" (7), is recognized by an enzyme moving only from right to left. In vivo, interaction with χ results in the stimulation of homologous recombination downstream of χ (69, 71). In vitro, recognition of the χ sequence causes RecBCD enzyme to switch the polarity of its nuclease activity (Fig. 4c): upon interaction with χ, degradation of the 3′-terminated strand is downregulated (24, 25), while degradation of the 5′-terminated strand is upregulated (2a). Consequently, the enzyme produces a lengthy ssDNA tail with χ at the 3′-terminated end. As determined from production of the χ-specific ssDNA fragments, the probability of recognizing a single χ is about 30 to 40% (3, 72). Interaction with χ also affects the helicase activity of RecBCD enzyme. Recognition of χ causes the enzyme to pause briefly (typically, a few seconds) at χ and to resume translocation after the χ site, but at a rate that is reduced by approximately twofold (68). Another consequence of χ modification is that the RecBCD enzyme gains the ability to "load" RecA protein onto the newly produced ssDNA (2b) (Fig. 4d). Thus, in response to χ, the RecBCD enzyme accomplishes both tasks essential for initiation of homologous recombination: (i) it recesses the DSB to produce an ssDNA-tailed duplex DNA with χ at its terminus, and (ii) it catalyzes formation of the RecA nucleoprotein filament on the ssDNA produced. This RecA nucleoprotein filament now can search for homology, promote invasion of the homologous recipient, and exchange the DNA strands (Fig. 4e).

The exact molecular mechanism by which χ recognition is translated into the observed changes in the activities of the RecBCD enzyme remains unknown. Most models propose either dissociation or inactivation of the RecD subunit (2, 19, 57, 73). Indeed, as mentioned previously, the RecBC enzyme (lacking the RecD subunit) is recombinationally proficient both in vivo (13) and in vitro (18). The RecBC enzyme is a processive helicase but with little or no nuclease activity (41), which is in contrast to the χ-activated form of RecBCD enzyme (Fig. 4a′); how-); however, this distinction is consistent with the requirement for RecJ nuclease activity in *recD* mutant cells in vivo (Fig. 4b′) (51). Similar to RecBCD enzyme, RecBC helicase facilitates asymmetric assembly of RecA protein only onto ssDNA that is 3′ terminal at the enzyme entry site (18) (Fig. 4e′). The RecBC-mediated loading of RecA protein is constitutive and is independent of χ, consistent with the phenotypic behavior of *recD* mutant cells in vivo.

REGULATED HELICASES/NUCLEASES IN OTHER BACTERIAL SPECIES

For decades, the interaction between RecBCD enzyme and χ was known to exist only in the species of enteric bacteria closely related to *E. coli*. But relatively recently, short (5 to 8 bp) sequences, which protect linear dsDNA from degradation by attenuation of the nuclease activity of RecBCD-like helicase/nuclease enzymes, were found in several distantly related bacteria (8, 16, 67). This finding indicates that, although apparently not universal, the regulation of recombinational helicases/nucleases by specific DNA sequences is widely spread among prokaryotes. While some bacteria possess clear homologues of RecBCD enzyme, other species contain its functional equivalent, the AddAB enzyme (reviewed in reference 15). AddAB helicase/nuclease comprises two subunits encoded by *addA* and *addB* genes. The sole motor subunit of AddAB enzyme, AddA protein, contains an SF1 helicase and a nuclease domain, which display a high degree of similarity to those in RecB protein (32). Also similar to RecB protein, AddA is a $3' \rightarrow 5'$ helicase, and its $3' \rightarrow 5'$ nuclease activity is downregulated upon interaction with the cognate

recombination hot spot of *Bacillus subtilis*, χ_{Bs} (5′-AGCGG-3′) (14, 16). The AddB subunit has no substantial similarity to either the RecC or the RecD subunit, but it does contain a putative ATPase motif and a second nuclease site similar to that of the AddA protein. The AddB subunit is responsible for the degradation of the 5′-terminated strand. Despite the limited sequence similarity to the RecBCD enzyme, AddAB enzyme is functional in *E. coli*: its expression overcomes the recombination and repair defects of *recBC*-deficient cells (40). Similar to RecBCD enzyme, AddAB enzyme binds to blunt-ended dsDNA, and uses the energy of ATP hydrolysis to translocate along and unwind dsDNA. However, whereas RecBCD enzyme degrades the dsDNA asymmetrically, the AddAB enzyme degrades both strands of the DNA duplex equally. Interaction with a correctly oriented χ_{Bs} results in downregulation of only the 3′ → 5′ nuclease activity of the translocating AddAB enzyme. The outcome, therefore, is the same as that occurring for the *E. coli* enzyme, namely, the production of ssDNA-tailed dsDNA with χ at the 3′ terminus. Homologues of AddAB enzyme are found in 12 different species of gram-positive bacteria, and χ homologues were identified in several bacterial species (reviewed in reference 15).

SINGLE-STRAND GAP REPAIR BY RECOMBINATION: THE RecF PATHWAY

The conjugal recombination deficiency of *recB* or *recC* mutants can be overcome by the combined effect of two extragenic suppressor mutations: *sbcB* and either *sbcC* or *sbcD* (suppressor of *recBC*). The *sbcB* mutation disables the nuclease activity of exonuclease I (44), while *sbcC* (31) disables one of the two subunits of the SbcCD nuclease, which, in wild-type *E. coli*, cleaves DNA hairpin and cruciform structures formed during replication of palindromic sequences (22, 49). The combined effect of these mutations is the full activation of an alternative pathway of sexual homologous recombination, referred to as the RecF pathway. Interestingly, the efficiency of conjugational and transductional recombination by the RecF pathway in the *recBC sbcBC* cells is similar to that of the RecBCD pathway in wild-type cells, showing that the machinery of this pathway can be as productive as that of the RecBCD pathway. Moreover, some bacterial species whose survival depends on homologous recombination (such as *Deinococcus radiodurans*) do not possess obvious RecBCD or AddAB enzymes, implying that a RecF-like pathway is the wild-type pathway in those bacteria.

Homologous recombination in the *recBC sbcB sbcC* mutant background depends on RecF, RecJ, RecN, RecO, RecQ, RecR, and SSB proteins. The processing of a DSB is likely achieved by the combined action of the RecQ helicase and RecJ nuclease. Although RecQ helicase is responsible for about 75% of conjugal recombination events occurring in *recBC sbcB sbcC* mutant cells, the remaining 25% require either UvrD (helicase II) or HelD (helicase IV) (53). The RecJ protein is an exonuclease that degrades ssDNA in the 5′ → 3′ direction (Fig. 5a to c). RecQ protein is an SF2 DNA helicase with a 3′ → 5′ polarity. Similar to RecBCD enzyme, RecQ helicase can unwind blunt-ended dsDNA (76). The helicase activity of RecQ protein is not limited to blunt dsDNA ends: the enzyme can also unwind an ssDNA-dsDNA junction with a 3′-ssDNA overhang (76), and even internal regions of dsDNA (33). It is hypothesized that DNA unwinding by RecQ helicase is coupled to the degradation of the 5′-terminated strand by RecJ nuclease, resulting in the production of the 3′-terminated ssDNA overhang, which can then be used as a substrate for RecA nucleoprotein assembly (Fig. 5c). In contrast to RecBCD enzyme, RecQ does not facilitate RecA nucleoprotein filament assembly. Therefore, the ssDNA produced by RecQ is bound by SSB protein and must be protected from degradation by nucleases. This explains the requirement for the *sbcB* mutation, since the preferred substrate for exonuclease I is SSB-complexed ssDNA.

Intriguingly, two major classes of mutations in the structural gene for exonuclease I were found. One class of mutations, referred to as *sbcB*, restores both recombination and the UV resistance of *recBC* cells. In contrast, the other class, known as *xonA* mutants, suppresses only the UV sensitivity but not the recombination deficiency of the *recBC* mutant bacteria (44). Exonuclease I activity is significantly reduced in both classes of mutants; moreover, UV sensitivity directly correlates with the amount of residual activity of the enzyme (60). The *sbcB* mutations are not the same as null mutations and, therefore, are likely gain-of-function mutations. The molecular mechanism distinguishing the two types of mutations still remains to be elucidated, but protection of the 3′ end of ssDNA by the *sbcB* mutations is envisioned.

The loading of RecA protein is an essential aspect of recombination in the RecBCD pathway (4). Not unexpectedly, the RecF pathway provides a RecA-loading activity in the form of the RecFOR complex. The genetic data had suggested that the loading of RecA protein onto SSB-coated ssDNA depends on the concerted action of RecF, RecO, and RecR proteins. First, mutant RecA proteins that

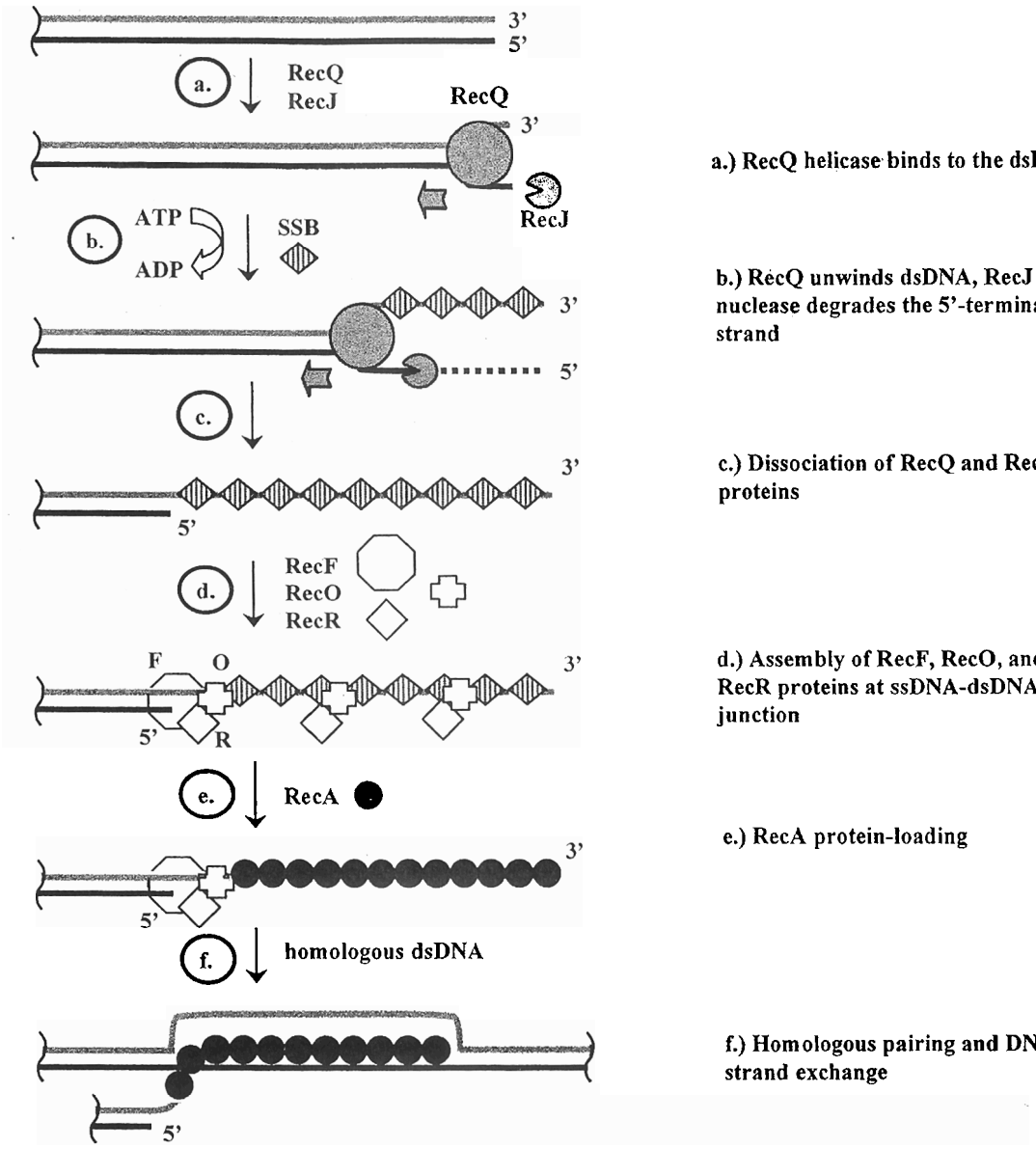

Figure 5. Initiation of homologous recombination at DSBs by the RecF pathway. Schematic representation of the early enzymatic steps of the RecF pathway. (a, b, and c) The combined action of RecQ helicase and RecJ nuclease converts a DSB into an ssDNA-dsDNA junction with 3′-terminated ssDNA overhang; this ssDNA is complexed with SSB protein. (d and e) The RecF, RecO, and RecR proteins form a complex at the junction and facilitate RecA nucleoprotein filament assembly on the SSB-coated ssDNA. (f) The RecA-ssDNA nucleoprotein filament invades homologous dsDNA.

suppress the UV sensitivity of *recF* mutations (such as the RecA803 protein) displace SSB protein from ssDNA much faster and more extensively than does the wild-type RecA protein (52), implying that RecF protein plays a role in SSB displacement. Second, a number of mutant *recA* alleles cosuppress mutations in *recF*, *recO*, and *recR* genes (79). Finally, this suppression is dependent on *recJ* function, suggesting that RecF, RecO, and RecR proteins function together as a complex (79). This view is strongly supported by biochemical observations showing that these proteins interact with one another (34, 74).

The RecFOR proteins form a number of complexes with different activities: RecO protein interacts with both RecR and SSB proteins (34) and facilitates RecA nucleoprotein filament assembly on SSB-coated ssDNA (75). RecO protein also promotes the annealing of ssDNA and of SSB-ssDNA complexes (38). Finally, RecR protein interacts with RecF protein to form a complex (34, 81) that will

bind to an ssDNA-dsDNA junction (55). Biochemical analysis revealed that RecF protein binds preferentially to the ssDNA-dsDNA junction (35), and that DNA binding by RecF protein is controlled by ATP hydrolysis (80). The RecFOR complex will bind to an ssDNA-dsDNA junction with a base-paired 5′ terminus at the junction region, and it will facilitate assembly of RecA protein onto ssDNA adjacent to the junction (55). RecF protein (or RecFR complex) recognizes an ssDNA-dsDNA junction with a 5′ end, which is the structure that should be produced by RecQ and RecJ proteins. The RecOR complex (or RecO protein) binds to the DNA-RecF(R) complex, which alters the SSB-ssDNA complex nearby and allows RecA protein nucleation; subsequent nucleoprotein filament extension permits assembly of RecA protein on the entire ssDNA tail.

Another component of the RecF pathway, RecN protein, has not yet been assigned any biochemical function. However, the slight recombination deficiency and mild UV sensitivity of the *recJ recN* double mutant, combined with the severe recombination defect (50- to 100-fold reduction) of the *recD recJ recN* mutant, suggests that RecN protein might be a functional equivalent of the RecJ nuclease (50).

Despite its apparent complexity, the enzymatic machinery of the RecF pathway is as functional in DSB repair as the RecBCD pathway. Moreover, the components of the RecF pathway have functional homologues or paralogues in all organisms, from bacteriophage to human (5).

SINGLE-STRAND GAP REPAIR

Because conjugation (and transduction) involves a DSB as the initiating site for recombination, it should not be surprising that the genes that emerged from a screen for mutants defective in sexual recombination are essential for the repair of DSBs. However, the consequence of this nearly singular focus on conjugational recombination events led to the erroneous conclusion that the RecF pathway serves only a minor function in recombination in wild-type cells. Also incorrect was the belief that the discovery of the RecF pathway as the set of genes that permitted recombination in the absence of the primary RecBCD pathway implied that the RecF pathway was a cryptic recombination pathway that could be activated to compensate for the loss of RecBCD enzyme function. Rather, in wild-type cells, the RecF pathway is responsible for the repair of all SSGs. This conclusion emerged from many studies, but was made clearest from recombination assays that did not employ sexual events.

A genetic assay system that observes recombination between direct repeats of a chromosomal segment allows following sister chromosome exchanges required for the repair of DSBs (29). This assay was developed to approximate the function of homologous recombination in the repair of the DNA lesions produced during replication. The recombination events occurring between direct chromosomal repeats are detected in the colony-sectoring recombination assay, since the detected recombination events eliminate the joint-point markers located between the repeats. Recombination events detected in this assay are absolutely dependent on RecA protein function. The *recF* and *recJ* mutants display a rec^+ phenotype, *recB* mutants show only a slight defect, and *recB recJ* double mutants are capable of supporting duplication segregation. On the other hand, *recB recF* double mutants are deficient in recombination between chromosomal direct repeats, suggesting that both RecBCD and RecF pathways play major roles in recombination.

Initiation of homologous recombination on SSGs is presented in Fig. 6. First, the RecFOR complex assembles at the ssDNA-dsDNA junction of an SSG containing a base-paired 5′ end (Fig. 6a) and facilitates RecA nucleoprotein assembly in the ssDNA region of the gap (55) (Fig. 6e). The RecFR complex can bind to the 3′-containing end of SSG, limiting RecA filament extension into the dsDNA region (82) (Fig. 6f). The resulting RecA nucleoprotein filament formed on the SSG can then invade the homologous dsDNA molecule (Fig. 6g). DNA strand exchange followed by heteroduplex extension results in the formation of two Holliday junctions. To complete the repair, RecA protein must be removed from the junctions, and the Holliday junctions themselves need to be resolved. The processing of a Holliday junction into mature recombinant molecules is achieved by the RuvA, RuvB, and RuvC proteins (see references 64 and 83 for reviews). The RuvA tetramer is a four-way junction-specific recognition protein that binds to the Holliday junction and induces the square planar conformation of this junction. The RuvB protein is a specialized translocation protein that can "pump" or move DNA through its circular hexameric active form. To catalyze branch migration, two hexameric rings of RuvB protein bind to opposing arms of the Holliday junction that was recognized by the RuvA proteins, and then they translocate the dsDNA outward through the center of each ring, resulting in the relative movement of the junction (Fig. 6h). RuvC protein is a four-way junction-specific endonuclease that resolves a Holliday junction by symmetrically cleaving the opposite arm of the junction to

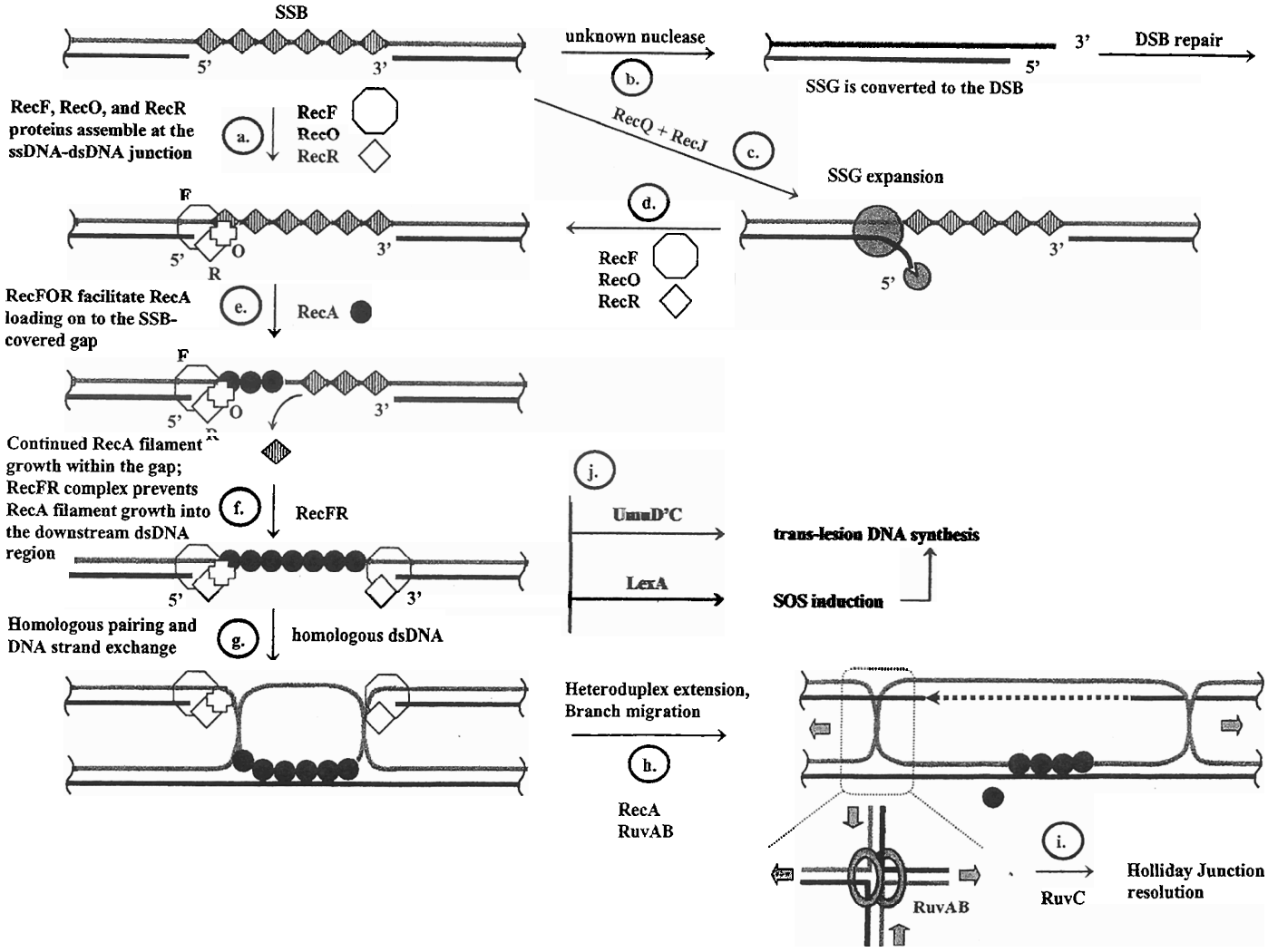

Figure 6. SSG repair by the RecF pathway. The SSG has several potential fates. The RecFOR proteins can bind to the 5′ end of the ssDNA-dsDNA junction (a), the SSG can be cleaved to produce a DSB (b), or the ssDNA region can be expanded by the combined activities of the RecQ helicase and RecJ nuclease (c and d). (e) RecFOR proteins facilitate RecA protein loading onto the SSB-coated ssDNA. (f) Growth of the RecA nucleoprotein filament beyond the ssDNA region is prevented by the RecFR complex bound to the ssDNA-dsDNA junction containing a free 3′ end. (g) The RecA nucleoprotein filament invades homologous dsDNA and catalyzes DNA strand exchange. (h) DNA heteroduplex expansion results in the formation of two Holliday junctions. (i) RuvABC proteins facilitate branch migration and Holliday junction resolution to produce repaired recombinant molecules. (j) Translesion DNA synthesis by DNA polymerase (UmuD′C) can also repair the SSG due to direct interaction of the polymerase with the RecA nucleoprotein filament.

produce the recombinant DNA product molecules (Fig. 6i).

Alternatively, an ssDNA endonuclease can convert the SSG into a DSB, which can be repaired through a DSB repair mechanism (Fig. 6b). If the region of ssDNA in the gap is too small, then it can be expanded by the combined action of RecQ helicase and RecJ nuclease. Similar to the DSB repair situation, RecQ helicase function can be substituted by UvrD helicase or helicase IV (53) (Fig. 6c). The SSG, on which the RecA nucleoprotein filament is assembled, is not necessarily repaired only by homologous recombination. The error-prone UmuD'C DNA polymerase can be attracted to such a RecA filament assembled on the SSG to catalyze translesion DNA synthesis (59) (Fig. 6j).

CONCLUSION

Homologous recombination can be initiated at either DSBs or SSGs in duplex DNA. Two major pathways are responsible for homologous recombination in wild-type *E. coli*: the RecBCD and RecF pathways. The RecBCD pathway is specific for the recombinational repair of DSBs, and in the wild-type cells, the RecF pathway is primarily used for recombination that initiates at SSGs. However, with appropriate suppressor mutations in *E. coli*, and presumably in bacteria that lack a RecBCD pathway, the RecF pathway can efficiently act at DSBs as well. Despite the different initiating lesions, both pathways have the same subsequent step: conversion of the broken DNA molecule into a central intermediate of recombination, which is the RecA protein nucleoprotein filament assembled along the ssDNA. In the RecBCD pathway, this process is carried out by the combined helicase/nuclease activity of RecBCD enzyme, and depends on the presence of the recombination hot spot, χ. In the RecF pathway, the combined efforts of RecQ helicase and RecJ nuclease are needed in combination with the RecFOR complex. In the RecBCD pathway, RecBCD enzyme facilitates assembly of the RecA protein onto SSB-coated ssDNA; in the RecF pathway, this task is accomplished by RecF, RecO, and RecR proteins. The RecA nucleoprotein filament can then initiate invasion of ssDNA into homologous dsDNA, progressing into the final stage of homologous recombination, which is resolution by RuvABC proteins.

REFERENCES

1. **Amundsen, S. K., A. F. Taylor, A. M. Chaudhury, and G. R. Smith.** 1986. *recD*: the gene for an essential third subunit of exonuclease V. *Proc. Natl. Acad. Sci. USA* 83:5558–5562.
2. **Anderson, D. G., J. J. Churchill, and S. C. Kowalczykowski.** 1997. Chi-activated RecBCD enzyme possesses $5' \rightarrow 3'$ nucleolytic activity, but RecBC enzyme does not: evidence suggesting that the alteration induced by Chi is not simply ejection of the RecD subunit. *Genes Cells* 2:117–128.

2a. **Anderson, D. G., and S. C. Kowalczykowski.** 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.

2b. **Anderson, D. G., and S. C. Kowalczykowski.** 1997. The translocating RecBCD enzyme stimulates recombination by directing RecA protein onto ssDNA in a χ-regulated manner. *Cell* 90:77–86.

2c. **Aravind, L., K. S. Makarova, and E. V. Koonin.** 2000. Holliday junction resolvases and related nucleases: identification of new families, phyletic distribution and evolutionary trajectories. *Nucleic Acids Res.* 28:3417–3432.

3. **Arnold, D. A., P. R. Bianco, and S. C. Kowalczykowski.** 1998. The reduced levels of χ recognition exhibited by the $RecBC^{1004}D$ enzyme reflect its recombination defect in vivo. *J. Biol. Chem.* 273:16476–16486.
4. **Arnold, D. A., and S. C. Kowalczykowski.** 2000. Facilitated loading of RecA protein is essential to recombination by RecBCD enzyme. *J. Biol. Chem.* 275:12261–12265.
5. **Beernink, H. T., and S. W. Morrical.** 1999. RMPs: recombination/replication mediator proteins. *Trends Biochem. Sci.* 24:385–389.
6. **Bianco, P. R., and S. C. Kowalczykowski.** 1999. RecA protein. In *Encyclopedia of Life Sciences*. [Online.] http://www.els.net. Nature Publishing Group, London, England.
7. **Bianco, P. R., and S. C. Kowalczykowski.** 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. USA* 94:6706–6711.
8. **Biswas, I., E. Maguin, S. D. Ehrlich, and A. Gruss.** 1995. A 7-base-pair sequence protects DNA from exonucleolytic degradation in Lactococcus lactis. *Proc. Natl. Acad. Sci. USA* 92:2244–2248.
9. **Blattner, F. R., G. Plunkett III, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao.** 1997. The complete genome sequence of Escherichia coli K-12. *Science* 277:1453–1474.
10. **Boehmer, P. E., and P. T. Emmerson.** 1992. The RecB subunit of the Escherichia coli RecBCD enzyme couples ATP hydrolysis to DNA unwinding. *J. Biol. Chem.* 267:4981–4987.
11. **Burland, V., G. Plunkett III, D. L. Daniels, and F. R. Blattner.** 1993. DNA sequence and analysis of 136 kilobases of the Escherichia coli genome: organizational symmetry around the origin of replication. *Genomics* 16:551–561.
12. **Capaldo-Kimball, F., and S. D. Barbour.** 1971. Involvement of recombination genes in growth and viability of *Escherichia coli* K-12. *J. Bacteriol.* 106:204–212.
13. **Chaudhury, A. M., and G. R. Smith.** 1984. A new class of Escherichia coli *recBC* mutants: implications for the role of recBC enzyme in homologous recombination. *Proc. Natl. Acad. Sci. USA* 81:7850–7854.
14. **Chédin, F., S. D. Ehrlich, and S. C. Kowalczykowski.** 2000. The Bacillus subtilis AddAB helicase/nuclease is regulated by its cognate Chi sequence in vitro. *J. Mol. Biol.* 298: 7–20.

15. **Chédin, F., and S. C. Kowalczykowski.** 2002. A novel family of regulated helicases/nucleases from Gram-positive bacteria: insights into the initiation of DNA recombination. *Mol. Microbiol.* **43**:823–834.
16. **Chédin, F., P. Noirot, V. Biaudet, and S. D. Ehrlich.** 1998. A five-nucleotide sequence protects DNA from exonucleolytic degradation by AddAB, the RecBCD analogue of Bacillus subtilis. *Mol. Microbiol.* **29**:1369–1377.
17. **Cheng, K. C., and G. R. Smith.** 1989. Distribution of Chi-stimulated recombinational exchanges and heteroduplex end-points in phage lambda. *Genetics* **123**:5–17.
18. **Churchill, J. J., D. G. Anderson, and S. C. Kowalczykowski.** 1999. The RecBC enzyme loads RecA protein onto ssDNA asymmetrically and independently of Chi, resulting in constitutive recombination activation. *Genes Dev.* **13**:901–911.
19. **Churchill, J. J., and S. C. Kowalczykowski.** 2000. Identification of the RecA protein-loading domain of RecBCD enzyme. *J. Mol. Biol.* **297**:537–542.
20. **Clark, A. J.** 1973. Recombination deficient mutants of E. coli and other bacteria. *Annu. Rev. Genet.* **7**:67–86.
21. **Clark, A. J., and A. D. Margulies.** 1965. Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12. *Proc. Natl. Acad. Sci. USA* **53**:451–459.
22. **Connelly, J. C., L. A. Kirkham, and D. R. Leach.** 1998. The SbcCD nuclease of Escherichia coli is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. *Proc. Natl. Acad. Sci. USA* **95**:7969–7974.
23. **Dillingham, M. S., M. Spies, and S. C. Kowalczykowski.** 2003. RecBCD enzyme is a bipolar DNA helicase. *Nature* **423**:893–897.
24. **Dixon, D. A., J. J. Churchill, and S. C. Kowalczykowski.** 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. USA* **91**:2980–2984.
25. **Dixon, D. A., and S. C. Kowalczykowski.** 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.
26. **Emmerson, P. T.** 1968. Recombination deficient mutants of *Escherichia coli* K12 that map between *thyA* and *argA*. *Genetics* **60**:19–30.
27. **Farah, J. A., and G. R. Smith.** 1997. The RecBCD enzyme initiation complex for DNA unwinding: enzyme positioning and DNA opening. *J. Mol. Biol.* **272**:699–715.
28. **Friedberg, E. C., G. C. Walker, and W. Siede.** 1995. *DNA Repair and Mutagenesis.* ASM Press, Washington, D.C.
29. **Galitski, T., and J. R. Roth.** 1997. Pathways for homologous recombination between chromosomal direct repeats in Salmonella typhimurium. *Genetics* **146**:751–767.
30. **Ganesan, S., and G. R. Smith.** 1993. Strand-specific binding to duplex DNA ends by the subunits of Escherichia coli recBCD enzyme. *J. Mol. Biol.* **229**:67–78.
31. **Gibson, F. P., D. R. Leach, and R. G. Lloyd.** 1992. Identification of sbcD mutations as cosuppressors of recBC that allow propagation of DNA palindromes in *Escherichia coli* K-12. *J. Bacteriol.* **174**:1222–1228.
32. **Haijema, B. J., G. Venema, and J. Kooistra.** 1996. The C terminus of the AddA subunit of the *Bacillus subtilis* ATP-dependent DNase is required for the ATP-dependent exonuclease activity but not for the helicase activity. *J. Bacteriol.* **178**:5086–5091.
33. **Harmon, F. G., and S. C. Kowalczykowski.** 2001. Biochemical characterization of the DNA helicase activity of the Escherichia coli RecQ helicase. *J. Biol. Chem.* **276**:232–243.
34. **Hegde, S. P., M. H. Qin, X. H. Li, M. A. Atkinson, A. J. Clark, M. Rajagopalan, and M. V. Madiraju.** 1996. Interactions of RecF protein with RecO, RecR, and single-stranded DNA binding proteins reveal roles for the RecF-RecO-RecR complex in DNA repair and recombination. *Proc. Natl. Acad. Sci. USA* **93**:14468–14473.
35. **Hegde, S. P., M. Rajagopalan, and M. V. V. S. Madiraju.** 1996. Preferential binding of *Escherichia coli* RecF protein to gapped DNA in the presence of adenosine (γ-thio) triphosphate. *J. Bacteriol.* **178**:184–190.
36. **Howard-Flanders, P., and L. Theriot.** 1966. Mutants of Escherichia coli K-12 defective in DNA repair and in genetic recombination. *Genetics* **53**:1137–1150.
37. **Iyer, V. N., and W. D. Rupp.** 1971. Usefulness of benzoylated naphthoylated DEAE-cellulose to distinguish and fractionate double-stranded DNA bearing different extents of single-stranded regions. *Biochim. Biophys. Acta* **228**:117–126.
38. **Kantake, N., M. V. Madiraju, T. Sugiyama, and S. C. Kowalczykowski.** 2002. Escherichia coli RecO protein anneals ssDNA complexed with its cognate ssDNA-binding protein: a common step in genetic recombination. *Proc. Natl. Acad. Sci. USA* **99**:15327–15332.
39. **Kogoma, T.** 1997. Stable DNA replication: interplay between DNA replication, homologous recombination, and transcription. *Microbiol. Mol. Biol. Rev.* **61**:212–238.
40. **Kooistra, J., B. J. Haijema, and G. Venema.** 1993. The Bacillus subtilis addAB genes are fully functional in Escherichia coli. *Mol. Microbiol.* **7**:915–923.
41. **Korangy, F., and D. A. Julin.** 1993. Kinetics and processivity of ATP hydrolysis and DNA unwinding by the RecBC enzyme from Escherichia coli. *Biochemistry* **32**:4873–4880.
42. **Kowalczykowski, S. C.** 2000. Initiation of genetic recombination and recombination-dependent replication. *Trends Biochem. Sci.* **25**:156–165.
43. **Krasin, F., and F. Hutchinson.** 1977. Repair of DNA double-strand breaks in *Escherichia coli*, which requires *recA* function and the presence of a duplicate genome. *J. Mol. Biol.* **116**: 81–98.
44. **Kushner, S. R., H. Nagaishi, and A. J. Clark.** 1972. Indirect suppression of *recB* and *recC* mutations by exonuclease I deficiency. *Proc. Natl. Acad. Sci. USA* **69**:1366–1370.
45. **Kuzminov, A.** 1995. Collapse and repair of replication forks in Escherichia coli. *Mol. Microbiol.* **16**:373–384.
46. **Kuzminov, A.** 1999. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage λ. *Microbiol. Mol. Biol. Rev.* **63**:751–813.
47. **Lam, S. T., M. M. Stahl, K. D. McMilin, and F. W. Stahl.** 1974. Rec-mediated recombinational hot spot activity in bacteriophage lambda. II. A mutation which causes hot spot activity. *Genetics* **77**:425–433.
48. **Lederberg, J., and E. L. Tatum.** 1953. Sex in bacteria; genetic studies, 1945–1952. *Science* **118**:169–175.
49. **Lloyd, R. G., and C. Buckman.** 1985. Identification and genetic analysis of *sbcC* mutations in commonly used *recBC sbcB* strains of *Escherichia coli* K-12. *J. Bacteriol.* **164**:836–844.
50. **Lloyd, R. G., and C. Buckman.** 1991. Overlapping functions of *recD*, *recJ* and *recN* provide evidence of three epistatic groups of genes in Escherichia coli recombination and DNA repair. *Biochimie* **73**:313–320.
51. **Lloyd, R. G., M. C. Porton, and C. Buckman.** 1988. Effect of recF, recJ, recN, recO and ruv mutations on ultraviolet survival and genetic recombination in a recD strain of Escherichia coli K12. *Mol. Gen. Genet.* **212**:317–324.

51a. **Lovett, S. T., C. Luisi-DeLuca, and R. D. Kolodner.** 1988. The genetic dependence of recombination in *recD* mutants of *Escherichia coli*. *Genetics* 120:37–45.
52. **Madiraju, M. V. V. S., A. Templin, and A. J. Clark.** 1988. Properties of a mutant recA-encoded protein reveal a possible role for Escherichia coli recF-encoded protein in genetic recombination. *Proc. Natl. Acad. Sci. USA* 85:6592–6596.
53. **Mendonca, V. M., H. D. Klepin, and S. W. Matson.** 1995. DNA helicases in recombination and repair: construction of a Δ*uvrD* Δ*helD* Δ*recQ* mutant deficient in recombination and repair. *J. Bacteriol.* 177:1326–1335.
54. **Michel, B., S. D. Ehrlich, and M. Uzest.** 1997. DNA double-strand breaks caused by replication arrest. *EMBO J.* 16: 430–438.
55. **Morimatsu, K., and S. C. Kowalczykowski.** 2003. RecFOR proteins load RecA protein onto gapped DNA to accelerate DNA strand exchange: a universal step of recombinational repair. *Mol. Cell* 11:1337–1347.
56. **Mosig, G.** 1987. The essential role of recombination in phage T4 growth. *Annu. Rev. Genet.* 21:347–371.
57. **Myers, R. S., A. Kuzminov, and F. W. Stahl.** 1995. The recombination hot spot χ activates RecBCD recombination by converting *Escherichia coli* to a *recD* mutant phenocopy. *Proc. Natl. Acad. Sci. USA* 92:6244–6248.
58. **Myers, R. S., and F. W. Stahl.** 1994. Chi and the RecBCD enzyme of Escherichia coli. *Annu. Rev. Genet.* 28:49–70.
59. **Pham, P., E. M. Seitz, S. Saveliev, X. Shen, R. Woodgate, M. M. Cox, and M. F. Goodman.** 2002. Two distinct modes of RecA action are required for DNA polymerase V-catalyzed translesion synthesis. *Proc. Natl. Acad. Sci. USA* 99:11061–11066.
60. **Phillips, G. J., D. C. Prasher, and S. R. Kushner.** 1988. Physical and biochemical characterization of cloned *sbcB* and *xonA* mutations from *Escherichia coli* K-12. *J. Bacteriol.* 170:2089–2094.
61. **Phillips, R. J., D. C. Hickleton, P. E. Boehmer, and P. T. Emmerson.** 1997. The RecB protein of Escherichia coli translocates along single-stranded DNA in the 3′ to 5′ direction: a proposed ratchet mechanism. *Mol. Gen. Genet.* 254:319–329.
62. **Roman, L. J., A. K. Eggleston, and S. C. Kowalczykowski.** 1992. Processivity of the DNA helicase activity of *Escherichia coli* recBCD enzyme. *J. Biol. Chem.* 267:4207–4214.
63. **Roman, L. J., and S. C. Kowalczykowski.** 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.
64. **Shinagawa, H., and H. Iwasaki.** 1995. Molecular mechanisms of Holliday junction processing in Escherichia coli. *Adv. Biophys.* 31:49–65.
65. **Sinden, R. R., and R. S. Cole.** 1978. Repair of cross-linked DNA and survival of *Escherichia coli* treated with psoralen and light: effects of mutations influencing genetic recombination and DNA metabolism. *J. Bacteriol.* 136:538–547.
66. **Smith, C. L., J. G. Econome, A. Schutt, S. Klco, and C. R. Cantor.** 1987. A physical map of the Escherichia coli K12 genome. *Science* 236:1448–1453.
67. **Sourice, S., V. Biaudet, M. El Karoui, S. D. Ehrlich, and A. Gruss.** 1998. Identification of the Chi site of Haemophilus influenzae as several sequences related to the Escherichia coli Chi site. *Mol. Microbiol.* 27:1021–1029.
68. **Spies, M., P. R. Bianco, M. S. Dillingham, N. Handa, R. J. Baskin, and S. C. Kowalczykowski.** 2003. A molecular throttle: the recombination hotspot, χ, controls DNA translocation by the RecBCD helicase. *Cell* 114:647–654.
69. **Stahl, F. W., J. M. Crasemann, and M. M. Stahl.** 1975. Rec-mediated recombinational hot spot activity in bacteriophage lambda. III. Chi mutations are site-mutations stimulating rec-mediated recombination. *J. Mol. Biol.* 94:203–212.
70. **Stahl, F. W., K. D. McMilin, M. M. Stahl, J. M. Crasemann, and S. Lam.** 1974. The distribution of crossovers along unreplicated lambda bacteriophage chromosomes. *Genetics* 77:395–408.
71. **Stahl, F. W., and M. M. Stahl.** 1975. Rec-mediated recombinational hot spot activity in bacteriophage lambda. IV. Effect of heterology on Chi-stimulated crossing over. *Mol. Gen. Genet.* 140:29–37.
72. **Taylor, A. F., D. W. Schultz, A. S. Ponticelli, and G. R. Smith.** 1985. RecBC enzyme nicking at Chi sites during DNA unwinding: location and orientation-dependence of the cutting. *Cell* 41:153–163.
73. **Taylor, A. F., and G. R. Smith.** 1999. Regulation of homologous recombination: Chi inactivates RecBCD enzyme by disassembly of the three subunits. *Genes Dev.* 13:890–900.
74. **Umezu, K., N. W. Chi, and R. D. Kolodner.** 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. USA* 90:3875–3879.
75. **Umezu, K., and R. D. Kolodner.** 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.
76. **Umezu, K., K. Nakayama, and H. Nakayama.** 1990. Escherichia coli RecQ protein is a DNA helicase. *Proc. Natl. Acad. Sci. USA* 87:5363–5367.
77. **Wang, J., R. Chen, and D. A. Julin.** 2000. A single nuclease active site of the Escherichia coli RecBCD enzyme catalyzes single-stranded DNA degradation in both directions. *J. Biol. Chem.* 275:507–513.
78. **Wang, T. C., and S. H. Chen.** 1992. Similar-sized daughter-strand gaps are produced in the leading and lagging strands of DNA in UV-irradiated E. coli uvrA cells. *Biochem. Biophys. Res. Commun.* 184:1496–1503.
79. **Wang, T.-C. V., H. Y. Chang, and J. L. Hung.** 1993. Cosuppression of *recF*, *recR* and *recO* mutations by mutant *recA* alleles in *Escherichia coli* cells. *Mutat. Res.* 294:157–166.
80. **Webb, B. L., M. M. Cox, and R. B. Inman.** 1999. ATP hydrolysis and DNA binding by the Escherichia coli RecF protein. *J. Biol. Chem.* 274:15367–15374.
81. **Webb, B. L., M. M. Cox, and R. B. Inman.** 1995. An interaction between the Escherichia coli RecF and RecR proteins dependent on ATP and double-stranded DNA. *J. Biol. Chem.* 270:31397–31404.
82. **Webb, B. L., M. M. Cox, and R. B. Inman.** 1997. Recombinational DNA repair: the RecF and RecR proteins limit the extension of RecA filaments beyond single-strand DNA gaps. *Cell* 91:347–356.
83. **West, S. C.** 1996. The RuvABC proteins and Holliday junction processing in *Escherichia coli*. *J. Bacteriol.* 178:1237–1241.
84. **Willetts, N. S., and D. W. Mount.** 1969. Genetic analysis of recombination-deficient mutants of *Escherichia coli* K-12 carrying *rec* mutations cotransducible with *thyA*. *J. Bacteriol.* 100:923–934.
85. **Yu, M., J. Souaya, and D. A. Julin.** 1998. Identification of the nuclease active site in the multifunctional RecBCD enzyme by creation of a chimeric enzyme. *J. Mol. Biol.* 283:797–808.