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RECOMBINATIONAL DNA REPAIR OF DAMAGED REPLICATION FORKS IN *ESCHERICHIA COLI*: Questions

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■ **Abstract** It has recently become clear that the recombinational repair of stalled replication forks is the primary function of homologous recombination systems in bacteria. In spite of the rapid progress in many related lines of inquiry that have converged to support this view, much remains to be done. This review focuses on several key gaps in understanding. Insufficient data currently exists on: (*a*) the levels and types of DNA damage present as a function of growth conditions, (*b*) which types of damage and other barriers actually halt replication, (*c*) the structures of the stalled/collapsed replication forks, (*d*) the number of recombinational repair paths available and their mechanistic details, (*e*) the enzymology of some of the key reactions required for repair, (*f*) the role of certain recombination proteins that have not yet been studied, and (*g*) the molecular origin of certain in vivo observations associated with recombinational DNA repair during the SOS response. The current status of each of these topics is reviewed.

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INTRODUCTION

Homologous genetic recombination is a substantial part of the molecular basis of the science of genetics. However, it has been clear for decades that homologous genetic recombination systems did not evolve to generate genetic diversity in populations or to assist geneticists in their efforts to map genes. A primary role in DNA repair has been the major alternative hypothesis (11, 12, 22, 26, 29, 36, 44, 67, 88, 100, 122). This view has now been refined. The major function of homologous genetic recombination in bacteria, and a major function in virtually all cells, is the nonmutagenic recombinational DNA repair of stalled or collapsed replication forks. This hypothesis is built on a recent convergence of many independent lines of research (28–32, 64, 67–69, 97, 98).

The DNA metabolism of every cell is replete with connections between replication and recombination. Replication is part of the recombination that accompanies bacterial conjugation or transduction (149, 150). In bacteriophage T4, the two processes are tightly linked, with recombination essential to the process of replication initiation after the first few replication cycles (82, 108, 109). In eukaryotes, replication accompanies the repair of programmed double-strand breaks in meiosis and the miscellaneous double-strand breaks that may occur as a result of exposure to ionizing radiation (46). In some cases, break (recombination)-induced replication can replicate major parts of a chromosome (15, 85, 106, 166) or permit telomere maintenance in eukaryotic cells lacking telomerase (15, 71).

The repair of replication forks is more than just another process to fill out this list. In bacteria, replication forks appear to require recombinational DNA repair often under normal growth conditions. Best current estimates indicate that a replication fork undergoes such repair in nearly every cell in every generation, making this the most important bacterial application of homologous genetic recombination on a frequency-of-use basis (29, 31, 32). In mammals, perhaps ten forks undergo recombinational DNA repair in every mitotic division (46). The need for nonmutagenic repair when replication forks encounter template damage provides an excellent rationale for the evolution of recombination systems. From this standpoint, the repair of replication forks might be considered the original or determinative function of homologous genetic recombination. The systems that have evolved in bacteria (and in eukaryotes, where many enzymes probably remain to be discovered) are elaborate and often redundant. Subsets of the recombination enzymes provide the potential for an adaptable response to whatever DNA structure is found at a stalled fork. Recombination during conjugation or transduction must then be considered a byproduct of the presence of this repair system.

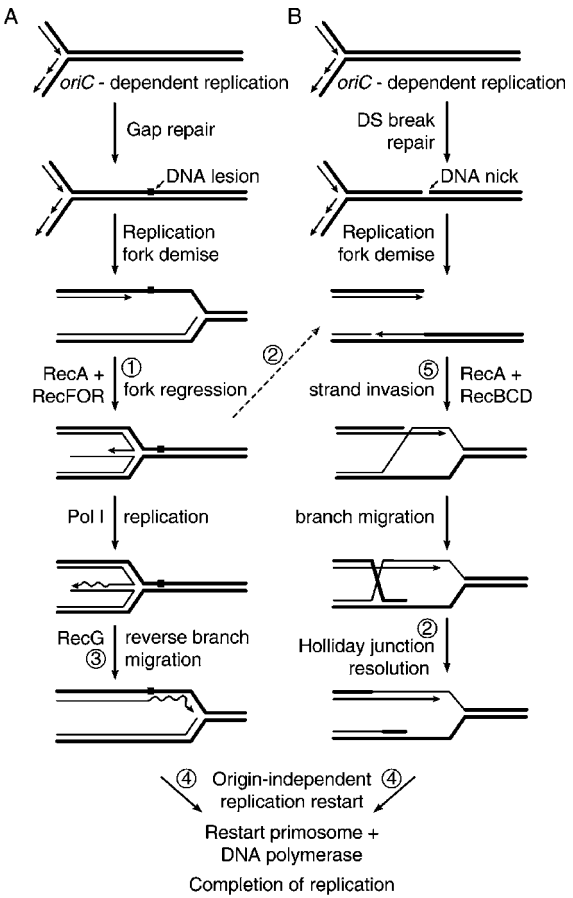

Figure 1 Pathways for the recombinational DNA repair of stalled or collapsed replication forks. The path in column *A* describes some of the processes proposed for the repair of forks that encounter an unrepaired DNA lesion. Column *B* illustrates some of the steps in the repair of double-strand breaks resulting from the encounter of a fork with a template strand break. Circled numbers correspond to five key processes described in the text.

There are five major types of reactions that underlie most of the proposed pathways for fork repair (Figure 1). If a fork encounters one of the spontaneous DNA lesions that occur in every cell (3000–5000 lesions per bacterial cell per generation under normal aerobic growth conditions), replication may halt and leave the lesion in a single-strand gap (Figure 1*A*). Four of the basic reactions can be seen in the possible pathways for repair of such a gap. First, fork regression involves the re-pairing of the DNA template strands such that the fork is moved backwards. The nascent DNA strands are eventually paired to generate a Holliday

structure with one abbreviated branch. Such structures have been observed for nearly 30 years and have recently been labeled "chicken feet" (121). Second, the Holliday structure can be cleaved by the RuvC or a related enzyme. This leads to a double-strand break. Third, as an alternative to cleavage, the structure can be subjected to enzymatic branch migration. In principle, this can move the branch back toward the original point where replication stalled, or further regress the fork. Fourth, all recombinational repair processes must include replication restart.

Many of the same processes can be seen in the likely pathways for repairing a double-strand break (Figure 1*B*). In addition, there is one new process, the invasion of the 3′ end of a single-stranded DNA into a homologous duplex DNA to generate a branched intermediate that can be processed to a form compatible with replication restart (#5 in Figure 1*B*). This must be considered one of the central reactions in recombination, playing a role in the repair of free DNA ends in all organisms in many contexts. This aspect of replication fork repair gives rise to key steps in conjugation and transduction in bacteria, and meiotic recombination in eukaryotes. Once invasion has occurred, the now paired 3′ end can be utilized as a primer for replication.

The new focus on replication fork repair is a significant paradigm shift within the broader study of DNA metabolism, and an illuminating one. Replication fork repair can take its place with excision repair, mismatch repair, base excision repair, and direct repair [such as the reaction catalyzed by DNA photolyase (135)] as a major cellular DNA repair pathway. When placed in this context, fork repair is readily seen as the least understood of the major cellular DNA repair processes.

Genetic recombination has often been viewed as a way to generate genetic diversity (thus altering genomes), but the application of recombination systems to replication fork repair provides another perspective. The recombinational repair of replication forks functions to maintain genome integrity. Most DNA repair processes (such as excision repair) are enabled by the double-stranded character of DNA. When a lesion occurs in one strand, it can be cut out. The other strand can be used as a template for replication to replace the damaged strand with correct genomic information. Replication fork encounters with strand breaks or lesions tend to generate structures in which both DNA strands are damaged. When a replication fork encounters a DNA lesion, the lesion is left in a single-strand gap. The recombination steps in Figure 1*A* do not directly repair the DNA lesion, but instead create the situation (an undamaged complementary strand) needed to effect later repair. When a replication fork encounters a template strand break, the recombination steps (Figure 1*B*) can be more readily seen as a true repair process designed to reconstruct the fork and permit a restart of replication. In either case, the recombination process is needed to permit a continuation of replication without introducing alterations in the DNA.

Many different enzymes participate in recombinational DNA repair. When DNA degradation processes and the action of a variety of enzymes with ATPase

activities are factored in, the repair of one stalled replication fork consumes a considerable amount of chemical energy in the form of expended dNTPs and rNTPs. Such energetic expenditures are a trademark of DNA repair systems such as mismatch repair (102) and the direct repair of O^6-alkylguanine lesions (118), presumably reflecting the generally low tolerance of biological systems for genome damage.

Our current understanding of nonmutagenic replication fork repair has been detailed in a number of recent reviews (30–32, 64, 68, 69, 97). This review attempts to explore some of the more impressive gaps in understanding, focusing entirely on the fork repair process in bacteria.

SPONTANEOUS DNA DAMAGE

Replication fork repair is probably most often predicated by a collision of a fork with DNA damage. The precise path taken by the repair process may depend to a large extent on what type of damage is encountered and whether it is on the leading or lagging strand template. Surprisingly little information is available about DNA damage frequencies in bacterial cells under different sets of growth conditions. Estimates of the spontaneous frequency of events such as depurination and cytosine deamination are readily available (43, 74). However, the majority of DNA lesions that occur spontaneously in a cell growing aerobically are oxidative lesions, arising from the action of hydroxyl radicals (57, 153). Indeed, many normally inviable cells lacking certain combinations of replication and recombination functions are able to grow anaerobically (52, 75, 104).

Oxidative damage includes a wide range of lesions, not all of which have been characterized. Under normal aerobic growth conditions, the best numbers available suggest that an *Escherichia coli* culture suffers 3000–5000 DNA lesions per cell per generation. Spent culture media from an *E. coli* culture harvested at an $A_{600} = 1.0$ yields sufficient 8-oxo-7,8-dihydro-2′-deoxyguanosine (oxo8dG) to account for several hundred of the corresponding lesions per cell per generation (116). Other work indicates that oxo8dG represents about 5% of the oxidative lesions in a typical spectrum of oxidative damage (126), providing the final estimate cited above. More detailed estimates that quantify the occurrence of a broader range of lesions under a variety of growth conditions would be very useful.

The potential barriers to replication forks do not begin and end with DNA lesions themselves, but include bound proteins, unusual DNA structures, and natural replication pause sites in the DNA (51, 97, 130). Recent studies suggest that RNA polymerase complexes themselves stalled at the sites of DNA lesions may be important barriers to replication in cells that have been irradiated with UV light (92). In general, a complete understanding of replication fork repair pathways will not be possible without a more comprehensive understanding of the situations in which replication forks are halted.

HOW OFTEN IS THE FORK HALTED?

Some controversy has persisted in the recombinational DNA repair literature concerning the capacity of replication forks to bypass DNA lesions. Bypass could take at least two forms. In some instances, the polymerase may insert a nucleotide opposite the lesion, potentially creating a mutation at that position. One can consider lesions as being of two types. Certain base analogues may cause mispairing and thus replication errors, but not block replication. These can include some naturally occurring lesions such as O^6-methylguanine. Lesions causing significant distortion in the DNA will instead block the progress of the replication fork. If the polymerase halts at the site of a lesion, replication might be restarted upstream by the same or a reconstituted DNA polymerase, leaving the lesion in a single-strand gap. In principle, this might occur more often on the lagging than the leading strand (94, 95) (Figure 2).

Much of the work to date on the effects of DNA lesions on replication has focused on UV-irradiated cells. A transient halt or inhibition of DNA replication can be readily observed in irradiated bacterial cells (131). The inhibition is amplified if the cells to be irradiated lack the uvrA gene function and thus cannot make use of DNA excision repair. A similar phenomenon is seen in mammalian cells (16, 72). Sedimentation of chromosomal DNA following UV irradiation provided some early evidence that gaps appeared in the DNA at the sites of UV lesions (16, 72, 73, 131, 132). This in turn suggested that DNA synthesis continued upstream of the lesion following a short lag, and models for the repair of the resulting gaps were constructed based on that assumption (174). Later work demonstrated that RecA protein was directly required in some capacity to restart replication after UV irradiation (58, 179). A need for recombination functions to get significant

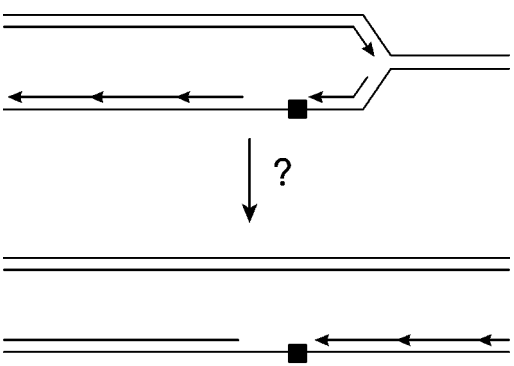

Figure 2 A potential path for bypass of DNA lesions on the lagging strand template of the replication fork. If the replication complex stays intact, it might be possible to abandon the synthesis of DNA at the lesion and revert to the synthesis of newly primed Okazaki fragments.

replication in the irradiated cells was also evident in the original experiments of Howard-Flanders and colleagues (131). This suggests that replication might proceed only after recombinational DNA repair was completed. It is well established that a variety of lesions, and particularly cyclobutane pyrimidine dimers, effectively halt DNA replication in vivo and in vitro (7, 9, 56, 70, 103, 123, 133, 155). Some replicational bypass of pyrimidine dimers in vitro by bacterial DNA polymerase III has been observed (77), but it is unclear if this would occur in a fully coupled replication fork or in vivo.

How and when replication restart can occur is still controversial, in spite of much research. Additional work is needed to determine how often (if ever) and under what circumstances replication can continue downstream of a lesion without being preceded by recombinational DNA repair. The pathway for recombinational repair of DNA gaps proposed by Howard-Flanders and coworkers required a nuclease activity to create the DNA ends needed for productive recombinational exchanges (174). The required nuclease has never been identified, although a candidate activity has been detected in bacterial extracts (19).

THE REPLICATION FORK TRAIN WRECK

Certain of my colleagues are fond of using photos of old train wrecks to accompany their descriptions of what occurs when a replication fork encounters DNA damage in one of the template strands. Unfortunately, these photos provide too apt a summary of the current understanding of the structure of forks following these encounters. There are multiple questions here, including the DNA structures present and the status of the replication enzymes.

There are only three instances in the current literature in which a stalled replication fork has been carefully characterized (Figure 3). Cordeiro-Stone and colleagues examined the fate of SV40 replication forks when they encountered pyrimidine dimers on the leading strand in vitro (25). Most of the stalled and deproteinized forks examined by electron microscopy exhibited the structure shown in Figure 3*A*. The leading strand synthesis had been halted at the site of the lesion, but the lagging strand had continued, perhaps uncoupled. The resulting fork typically had a leading strand gap on the order of 1000–2000 bases in length. This particular structure has been used as a starting point in a number of proposed models for fork repair, and is used in Figure 1*A*. The general structure of this stalled fork is compatible with stalled eukaryotic replication forks characterized by more indirect methods. Several additional studies of replication in cell-free extracts of mammalian cells produced encounters with a pyrimidine dimer or other bulky lesion, leading to the uncoupling of leading and lagging strand DNA synthesis and selective replication of the lagging strand (154, 157, 158). Another type of fork structure was found by Sogo and colleagues, who examined the structure of forks stalled at natural replication fork barriers near the rRNA gene clusters in vivo in yeast (45). In this case, the stalled fork also featured a lagging strand that had been completed ahead

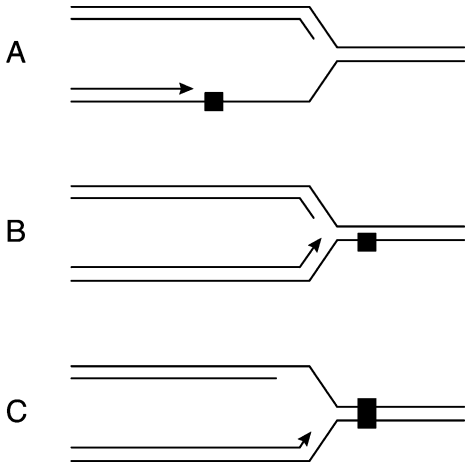

Figure 3 Characterized structures of stalled replication forks. *A.* The major structure found after an SV40 replication fork encounters a cyclobutane pyrimidine dimer in the leading strand (25). The single-strand gaps found at the forks were typically on the order of 1000 nucleotides or more. *B.* Structure of replication forks stalled at the rRNA replication fork barrier in vivo in yeast (45). Here, the newly synthesized lagging strand typically extends just a few nucleotides beyond the leading strand. *C.* Structure of a bacterial replication fork stalled at a replication termination site embedded in a plasmid substrate in vitro (49). The nascent leading strand extended 50–70 nucleotides beyond the lagging strand in these structures.

of the leading strand progress, but in this case the difference was only a few base pairs (Figure 3*B*). In the final instance, Hill & Marians examined the structure of forks stalled in vitro at a terminator site (ter), embedded in a plasmid template and bound with the terminator protein Tus (49). Here, the leading strand was ahead of the lagging strand, leaving a gap of 50–70 nucleotides in the lagging strand branch (Figure 3*C*).

If this handful of studies is any indication, the structures present at stalled forks are likely to be varied. The particular structures present are also likely to depend upon what sort of lesion or other barrier is encountered and the template strand (leading or lagging) it is found on. Since the stalled fork structure represents the starting point for any repair process, a better understanding of the diversity of structures that occur is a prerequisite to a full appreciation of the diversity in repair mechanisms.

In addition to the DNA structures present, it is also necessary to determine the disposition of replication enzymes after an encounter with an unreplicable barrier. It has not been determined if the DNA polymerase complexes disassemble, and which barriers trigger what degree of disassembly. A transition presumably occurs here where the replication complex gives way to recombination enzymes. The

mechanistic details of this transition will depend on what initially happens to the replication complex.

It is conceivable that certain proteins facilitate the replication to recombination transition. The RecF and RecR proteins might be good candidates for such transition mediator functions. In vitro, these proteins can bind to dsDNA as a complex and can block the extension of assembling RecA filaments (171, 172). This has suggested a role in modulating RecA filament formation. However, random binding to dsDNA in vivo would be unlikely to permit useful interaction with RecA filaments, and localization to stalled replication forks would require interaction with other proteins, perhaps the replication complex itself (172). Both proteins are co-transcribed with DNA polymerase subunits (13, 41, 119), and additional roles in recombinational DNA repair may remain to be elucidated.

RECOMBINATION PATHWAYS

The pathways outlined in Figure 1 are oversimplified, and a number of additional pathways or pathway variations have been proposed. Many of the variants begin with fork regression (process 1 in Figure 1*A*) and differ primarily in the starting point for regression and/or fate of the resulting "chicken foot."

Cells with mutations in certain replication functions are often dependent on recombination enzymes for survival and exhibit a hyperrec phenotype. This is one of the general observations linking recombination to replication fork repair (98). The replication defects that make bacterial growth recombination dependent are generally those that result in more frequent stalling of the replication fork.

Enzymes with defects in the accessory replicative helicase Rep make the cells dependent on a functional RecBCD enzyme, but not on RecA protein (140). A similar phenomenon is seen in cells with a mutation in the holD gene, which encodes the ψ subunit in the clamp-loading complex of DNA polymerase III (40). The properties of these mutant cells have given rise to a proposed pathway for fork repair in the absence of RecA protein. The tail produced by fork regression is degraded by RecBCD to generate a structure suitable for replication restart (Figure 4).

The chicken foot is a classical Holliday intermediate that can be processed by the RuvABC proteins. RuvA and RuvB form a complex that binds to a Holliday intermediate and promotes branch migration (173). The RuvC protein is a Holliday intermediate resolvase (173). The chicken foot intermediate can thus be cleaved to produce a double-strand break as shown in Figure 5. In *rep recBC* mutant cells, double-strand breaks accumulate (40, 130, 140). The appearance of these breaks depends on the presence of the RuvABC proteins, providing some of the evidence that the stalling of a replication fork can give rise to a Holliday intermediate (chicken foot) (40, 130, 140). This provides a pathway for the generation of double-strand breaks that is distinct from the double-strand break generation that occurs when replication forks encounter template strand breaks as demonstrated in vivo

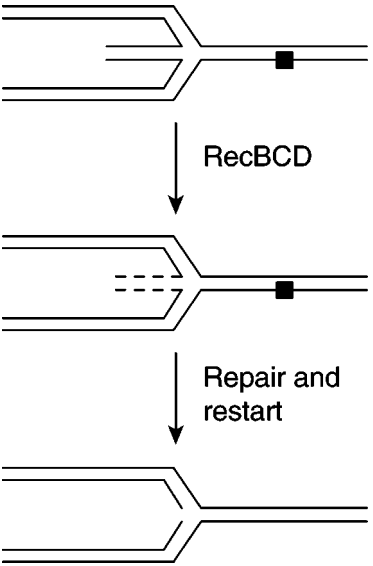

Figure 4 Pathway for the nucleolytic processing of a chicken foot structure by the RecBCD enzyme. The nuclease activity of RecBCD is proposed to eliminate the short arm of the structure, generating a structure that can be used to restart replication. If this pathway is to be successful, the lesion would have to be repaired by some process like excision repair while the fork was regressed and prior to replication restart.

by Kuzminov (69a) (Figure 1*B*). Regardless how they are generated, the repair of double-strand breaks requires the action of the RecBCD enzyme and the RecA protein. How replication fork repair events are distributed between pathways that do or do not involve double-strand breaks (Figure 1) is not known, and probably varies depending on factors such as growth conditions and the associated spectrum of spontaneous DNA damage.

While the list of potential repair pathways may already seem complicated, it is likely to grow. A determination of which repair pathways are most important may be difficult to achieve. A focus on one or a few pathways in individual research reports often reflects the particulars of the investigation. In bacterial cells growing aerobically, most of the replication forks undergo repair, with the potential for use of a wide range of repair pathways. Most in vivo studies directed at the elucidation of these repair paths make use of some strategy to amplify the signal by increasing the frequency of fork stalling. This may involve the use of replication mutants, or DNA-damaging treatments such as UV irradiation, or the use of growth conditions that lead to an increased number of replication barriers involving bound proteins. If the number of fork stalling events in the cell increases substantially, the cells may become completely dependent on the particular path that is used to bypass whatever barriers are presented to the replication forks, and the enzymes needed for

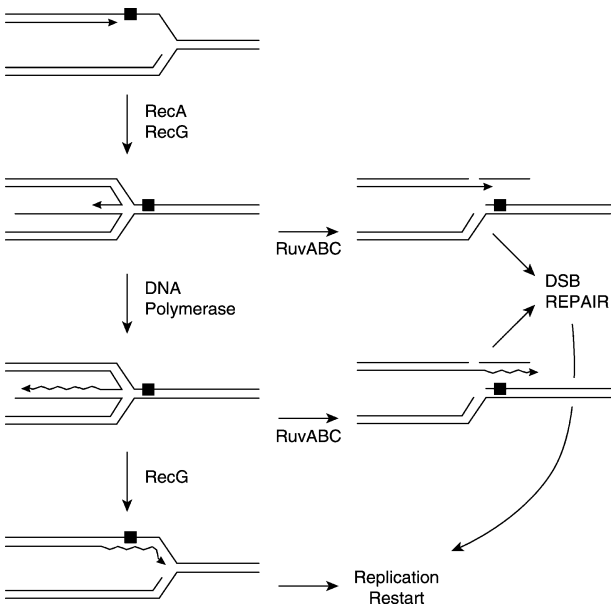

Figure 5 Some alternative paths for the processing of stalled replication forks. The chicken foot is a Holliday structure that can be processed and cleaved by the RuvABC enzymes. Cleavage at any stage funnels the process into the double-strand break repair pathway.

that path. Thus, individual in vivo research efforts have elucidated the molecular course of certain repair pathways, but generally shed little light on the question of which pathways are most important to the cell under normal growth conditions.

FIVE KEY REACTIONS IN THE RECOMBINATIONAL DNA REPAIR OF REPLICATION FORKS

Replication Fork Regression

Fork regression [also called fork reversal (98)] involves the re-pairing of the recently replicated template strands at a stalled fork, such that the fork is moved backwards (Figure 6). The newly synthesized strands are displaced and then paired as the fork is regressed to create a 4-branched structure, the chicken foot. Evidence for replication fork regression has been available in the literature for nearly three decades. The first proposal of a fork regression process as a means of repairing a replication fork was made by Higgins and coworkers (48). Many other reports of regressed fork structures were made in the years following the work of Higgins (53, 111, 156, 170, 181), although the significance of the structures was not always

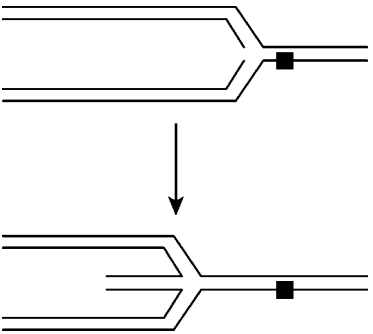

Figure 6 Replication fork regression to generate a chicken foot.

apparent. In bacteria, it is now clear that there are at least three paths to fork regression, and each of them may play a role in the repair of stalled replication forks. The simplest path is nonenzymatic. The positive supercoiling that builds up ahead of a replication fork will lead to spontaneous fork regression to produce a chicken foot structure (121). Such spontaneous fork regression during sample preparation may be the origin of some of the early reports of regressed forks. The spontaneous process may also play a direct role in repair. If a stalled fork undergoes regression, even limited to a few dozens or hundreds of base pairs, it can in principle provide loading sites for enzymes that bind to and process Holliday intermediates.

A second pathway for fork regression is provided by the activity of the RecG helicase. This enzyme is an ATPase and promotes branch migration at DNA junctions with 3 and 4 branches (79, 91, 92, 175). When a stalled fork has few or no single-strand gaps, this may be the principal enzymatic path to fork regression. Thus, RecG may be particularly important when replication forks are stalled by replication pause sites [such as the rDNA fork barriers in yeast (Figure 3*B*)] or bound proteins. Lloyd and coworkers have provided evidence that replication can be halted at bound RNA polymerases themselves stalled at DNA lesions, and that RecG protein plays a key role in the resulting fork repair process (92). More recent work establishes a role for RecG in the regression of forks stalled in vitro (93).

The final pathway for fork regression is provided by the RecA protein. If the stalled fork contains a substantial single-strand gap, such as a fork with the structure in Figure 3*A*, RecA protein can form a filament in the gap and promote fork regression. This reaction has been demonstrated in vitro and is quite efficient (127). RecA is a DNA-dependent ATPase, and RecA-promoted regression exhibits a requirement for ATP hydrolysis. When coupled to ATP hydrolysis, RecA protein–promoted DNA strand exchange reactions are unidirectional, with branch movement proceeding 5′ to 3′ relative to the bound single-stranded DNA in the gap

(55). If a RecA filament loads onto a leading strand gap such as that in Figure 3*A*, this direction of branch movement will result in fork regression. There is also in vivo evidence for a role of RecA in fork regression in some situations. The primary helicase at replication forks is the DnaB protein, and cells with a *dnaB*ts mutation exhibit high levels of fork stalling. The generation of recombination intermediates that can be processed by RuvABC (presumably chicken feet generated by fork regression) in these strains depends upon RecA function (141). However, it is as yet unclear whether the in vitro experiments demonstrating RecA-mediated fork regression (127) provide an appropriate model for the repair of stalled forks in *dnaB*ts cells. The DnaB helicase moves along the lagging strand template at the replication fork, and a defective helicase may cause fork stalling that leaves gaps opposite the lagging strand rather than the leading strand template (141). A RecA filament formed in a lagging strand gap would be expected to promote a DNA strand exchange in the direction opposite to that required for fork regression.

The in vitro efforts to date do not address the considerable topological complexities of the fork regression process. In the cell, fork regression is likely to require the action of helicases, topoisomerases, and other enzymes beyond those currently being investigated.

Holliday Intermediate Cleavage

The four-branched Holliday structure is a signature recombination intermediate, associated with a wide range of recombination processes. Enzymes that specifically cleave such structures to generate viable recombinant products have been found in many types of organisms (4). In *E. coli*, there are at least two such enzymes, RuvC and RusA (10, 18). The reaction generally involves symmetric cleavage of two opposing strands of the intermediate so that two branches remain with each product (Figure 7) (10). Thus, a given Holliday intermediate can be resolved in two ways, depending on which pair of strands is cleaved.

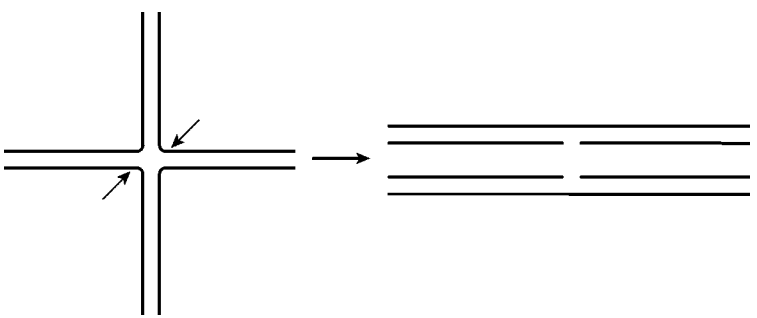

Figure 7 Cleavage of Holliday intermediates by resolvases like RuvC enzyme.

The resolution of Holliday intermediates can have interesting genomic consequences for the cell. If a Holliday intermediate is formed behind the replication fork during fork repair, cleavage of that intermediate can lead to the formation of chromosomal dimers (Figure 8). Such dimers occur in about 15% of all bacterial cells under normal growth conditions (152). Conversion of chromosomal dimers to monomeric chromosomes is the task of a specialized site-specific recombinase called the XerCD enzyme, acting at specific sites near the DNA replication termini (144). If XerCD is mutagenically inactivated, the unprocessed chromosomal dimers block cell division. Interestingly, the RuvABC proteins appear to bias the resolution of Holliday intermediates such that the chromosomal dimers formed in

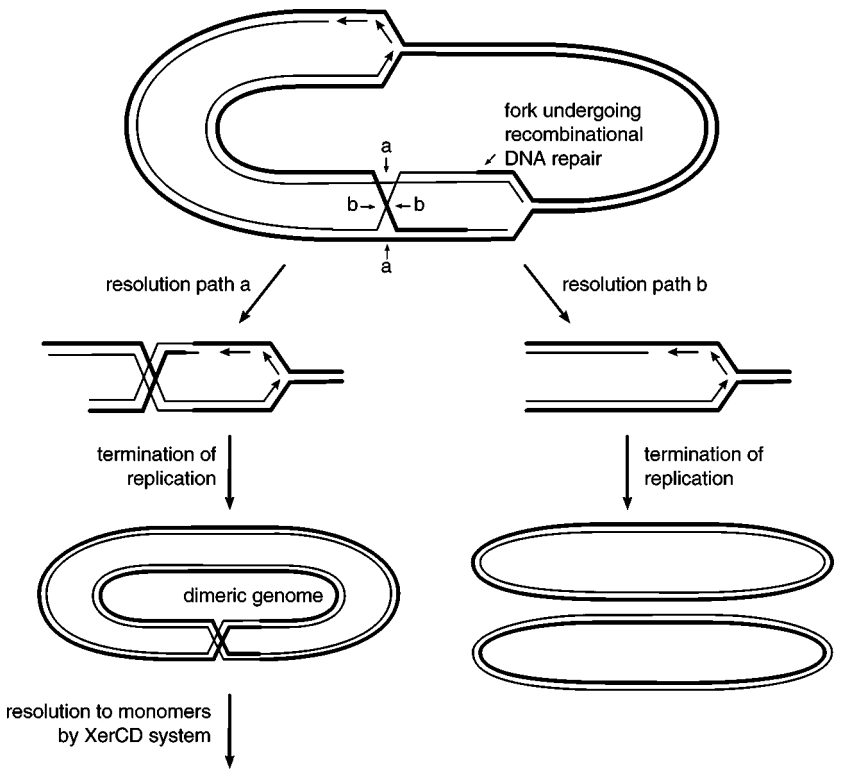

Figure 8 The generation of chromosome dimers by recombination and replication. In principle, cleavage of a Holliday intermediate placed behind the replication fork could occur in two ways, labeled *a* and *b*. Cleavage by pathway *a* generates a crossover, and the subsequent replication leads to the formation of a contiguous chromosomal dimer that must be converted to monomers by a specialized site-specific recombination system (XerCD).

15% of the cells represent less than half of the Holliday intermediate resolution events (8, 99). The mechanism by which that bias is preserved has been established (33, 99, 163).

Holliday Junction Branch Migration

This process is closely related to fork regression, but it deserves some additional discussion. The migration of DNA branches has been studied for several decades and has been a recognized part of recombination processes for a similar period of time. Spontaneous branch migration in isolate DNA proceeds in a random walk (112, 113, 159). With respect to replication fork repair, there is a need instead for directed branch migration. The fork regression process is one branch migration that can only proceed in one direction, and it can create a Holliday intermediate. Migration of the Holliday intermediate in the direction opposite to regression (or fork reversal; terminology gets tricky here as one begins to talk about the reverse of a reversal) can reset the replication fork (Figure 9). If the chicken foot structure is not cleaved, then this branch migration offers a conservative path to restoration of the replication fork.

There are at least three enzymes that might catalyze this process, RuvAB, RecG, and PriA. The RuvAB complex binds to a Holliday intermediate and promotes branch migration, with the direction of branch movement depending on the way RuvAB loads onto the DNA (117). In some reaction contexts, this loading appears to preferentially occur so as to reverse a DNA strand exchange reaction promoted by RecA protein (54). The RecG helicase has also been characterized as a branch migration activity (79, 175, 176), with a tendency to reverse RecA-mediated DNA strand exchange reactions in some reaction systems (175). RecA protein and either RuvAB or RecG could thus participate in a regression/reversal cycle, with intermediate replication and repair steps as needed to restore the fork. In some instances, RecG may act alone to promote both regression and its reversal (92). The PriA protein also has a helicase activity and may play a role in branch movement. There is some in vivo and in vitro evidence that PriA and RecG proteins may process branched structures in different ways (1, 90). One of the more substantial and interesting problems in fork repair will come in determining how these proteins interact and how their order and mode of action is determined to

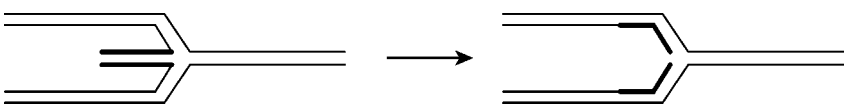

Figure 9 Resetting the replication fork by reverse branch migration in the chicken foot. The short branch of the chicken foot is shown with thick lines so that the fate of these strands can be seen in the product of the branch migration reaction.

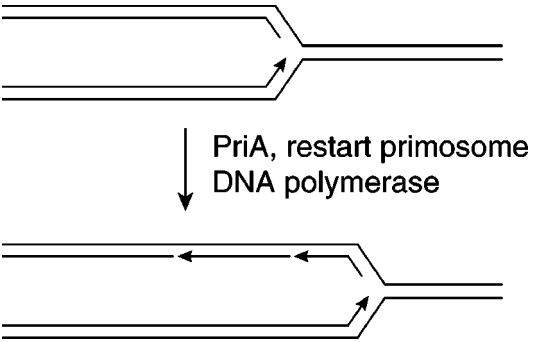

Figure 10 The origin-independent replication restart process makes use of branched recombination products.

bring about the end result of fork restoration. Additional proteins may well play a role in these molecular decisions.

Replication Restart Pathways

Every repaired replication fork must go through a restart process to resume replication (Figure 10). This process is by necessity origin-independent and distinct from the replication initiation that occurs at *oriC*. Origin-independent replication restart requires the activity of a seven-protein complex. This was originally known as the ϕX174-dependent primosome, reflecting the particulars of its discovery (147, 177) [see (31) for review], but has more recently been renamed the restart primosome (137). The assembly of this complex on certain types of recombination intermediates has been reconstituted in vitro (76, 87, 137). The entire process also must involve DNA polymerase III, and the cryptic DNA polymerase II may also play a role (124).

The restart primosome consists of seven proteins, with historical names given in parentheses: PriA (protein n′, factor Y), PriB (protein n), PriC (protein n′′), DnaT (protein i), DnaC, the DnaB helicase, and the DnaG primase. The PriA protein plays a key role in the assembly of the complex on a recombination intermediate (76). As might be expected if nearly all cells must go through fork repair and replication restart, cells lacking priA function are nearly inviable (86, 138). An unstated assumption that all fork restoration must go through a PriA-mediated restart process has served to link estimates of the frequency of cellular restart to the phenotype of priA mutant cells (29, 30, 32). Recently, evidence has appeared for the existence of priA-independent paths for replication restart (136). Thus, the frequency of replication fork stalling and recombinational DNA repair may be higher than the estimates drawn from the *priA* phenotype might suggest, perhaps involving several fork events per cell per generation.

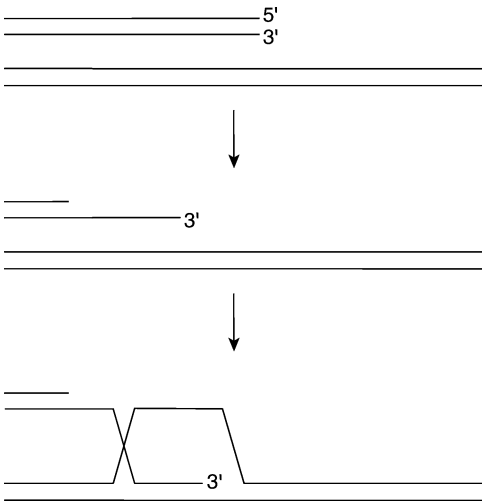

Figure 11 The DNA strand invasion reaction. In *E. coli*, the processing of the double-strand end in the first step is promoted by the RecBCD enzyme. The second step (invasion) is promoted by RecA protein.

3′ End Invasion

In some respects, this is the best-understood process within the broader topic of replication fork repair. The invasion of 3′ ends into a homologous duplex DNA (Figure 11) has been a key component of published recombination models since the early constructs of Meselson & Weigle (96). The process is central to the late stages of bacteriophage T4 replication (82, 108, 109), and it has been studied in some detail in vitro (66, 105). This same reaction was among the first reactions investigated in the early study of purified RecA protein (89, 145). When RecA protein is bound to linear single-stranded DNAs, there is a pronounced tendency for any subsequent invasion of a complementary duplex DNA to take place on the 3′ end of the single strand (37, 63). This reaction may be a necessary step in the repair path any time a replication fork branch is severed by a fork encounter with a strand break (Figure 1*B*) or a chicken foot repair intermediate is cleaved.

The inherent bias for 3′ ends in RecA-mediated strand invasion reactions comes about as a function of the directional bias observed in RecA filament formation and disassembly reactions. Once one or a few RecA monomers form a nucleus on a ssDNA molecule, filaments are extended 5′ to 3′ from that point (125, 142). Little addition of RecA monomers on the "wrong" 5′-proximal end can be detected (5). RecA filaments also disassemble in the 5′ to 3′ direction, such that monomers are added to and subtracted from filaments at opposite ends (5, 142). Both the assembly and disassembly processes help to assure that the 3′ ends of linear single-stranded DNAs are more likely to be bound by RecA protein, and thus to react in strand

invasion, than 5′ ends. The presence of SSB protein also helps ensure that RecA protein will not be present at the 5′ ends (5, 63, 142). Interestingly, the 3′ end bias is largely eliminated if the RecO and RecR proteins are included in the reaction mixture (J. Bork & M. Cox, unpublished observations). Possible functions for a RecAOR-mediated 5′ end invasion have not been explored.

The repair of double-strand breaks in *E. coli* generally represents a collaboration between the RecBCD enzyme and the RecA protein (11). *E. coli* mutant cells deficient in the activities necessary to mature Okazaki fragments on the lagging strand during replication (e.g., DNA ligase and DNA polymerase I) become completely dependent on the function of both the RecBCD enzyme and RecA protein (75, 101, 107, 169, 178) as a result of the many strand breaks present. The pathway leading to strand invasion has been reconstituted in vitro by Kowalczykowski and coworkers (3, 20). The RecBCD complex has both nuclease and helicase activities. RecBCD binds at a free duplex DNA end, simultaneously degrading and unwinding it. When it encounters an 8 nucleotide sequence called Chi, its DNA degradation properties are altered so that degradation of the 3′-ending strand is greatly reduced (2). The result is a processed end with a long single-strand extension with a 3′ end. The RecBCD protein plays a direct role in loading the RecA protein onto this single-strand extension, leading to strand invasion (3, 20).

Some interesting problems arise when an attempt is made to couple strand invasion to a restart of DNA synthesis. After RecA protein promotes DNA strand invasion, the continued presence of a RecA filament blocks access by DNA polymerases to the introduced 3′ end (K. Marians, personal communication). Extension of this 3′ end by any polymerase requires the prior disassembly of the RecA filament that created it. A complete reconstitution of DNA strand invasion with establishment of a complete replication fork should introduce many more interesting complexities.

THE ROLES OF MANY RECOMBINATION FUNCTIONS ARE IMPERFECTLY UNDERSTOOD

The recombinational DNA repair of stalled replication forks involves many proteins, and in many cases the activities of the proteins and their precise role in repair are not yet understood. I focus here only on a selection of proteins not mentioned in the preceding discussion. In the following section, gene map locations reflect the current published *E. coli* genome sequence (14).

The RadA protein (also called Sms; M_r 49,477, 99.7 min on the *E. coli* chromosome map) is encoded by a gene that exhibits homology to both the RecA protein and the lon protease (128). Mutations in the gene for RadA confer sensitivity to UV- and X-ray irradiation when the cells are grown in rich media (35, 151). Little is known about the activity of this protein, except that it appears to have a DNA-dependent ATPase activity (S. Lovett, personal communication). Defects in the RadC protein (M_r 25,573, 82.1 min) also confer sensitivity to UV and X rays in

rich media. RadC function is required to observe elevated levels of tandem repeat recombination induced by replication fork defects (139). There is thus a clear link between RadC and replication fork repair. Nothing is known about the molecular role of RadC protein.

The recX gene (also oraA) is located just downstream of the gene encoding the RecA protein and encodes a polypeptide with M_r 19,425, 60.8 min. The function of the RecX protein is not known, and the gene has been little studied. In other bacteria, a very closely related gene is found just downstream of the recA gene, and it is generally co-transcribed with recA (114, 115, 164). In *Streptomyces lividans*, the RecX protein function is required in cells in which RecA protein is overproduced, suggesting it moderates some toxic effect of RecA overexpression (164). The protein thus may interact with and perhaps regulate the RecA protein and/or the filaments it forms on DNA.

Somewhat more is known about some of the other auxiliary functions of recombinational DNA repair (65, 128). The RecF [M_r 40,518; 83.6 min (50)], RecO [M_r 27,393, 58.2 min (62)], and RecR [M_r 21,965, 10.6 min (83, 84)] proteins historically have helped define a recombination pathway distinct from that defined by the RecBCD enzyme (21, 22). The phenotypes of all three of these genes are quite similar, and there has been a general association between the proteins and the repair of DNA gaps (22). Additional in vivo data have suggested a direct interaction between these proteins and RecA protein. In particular, certain mutants of RecA protein (RecA441 and RecA803) suppress deficiencies in all three of the RecFOR functions (168). in vitro, the three proteins do not form a heterotrimer but instead form two pairwise RecOR and RecFR complexes. The activities of these proteins studied to date in vitro involve the modulation of RecA filament assembly and disassembly. The RecOR complex promotes RecA filament nucleation on ssDNA substrates that are already bound with the single-strand DNA binding protein (SSB) (160). The same complex also inhibits a net end-dependent disassembly of RecA filaments (142). The RecFR complex binds randomly to dsDNA and blocks the extension of growing RecA filaments (172). Together, these activities could suffice to constrain RecA filaments to DNA gaps where repair was to take place (172). However, RecOR is not needed for RecA function in fork regression either in vivo (141) or in vitro (127). As already noted, an interaction of RecFR with some component of the replication complex may be needed for this complex to function at the replication fork. Clearly, more remains to be discovered about the functions of these proteins.

Three other proteins have been loosely associated with the RecF pathway of recombination, although they may function in other contexts as well (22, 65). These are the RecJ [M_r 63,396, 65.4 min (80)], RecN [M_r 61,377, 59.3 min (78)], and RecQ proteins [M_r 68,441, 86.3 min (110)]. These three appear to be functionally quite distinct.

The RecJ protein is a 5′ to 3′ single-strand DNA exonuclease (81). The protein could play a variety of roles in the processing of displaced 5′ ends during recombination processes. RecJ can also play a role in DNA mismatch repair (24, 165).

Although initially associated with the RecF pathway, the properties of cells deficient in RecN function suggested a role in double-strand break repair (120). There are no reports of in vitro activities of RecN protein. This is primarily because the protein has proven to be insoluble and quite hard to work with. Partially purified preparations of RecN protein exhibit a weak ATPase activity and some nuclease activity, (T. Arenson & M. Cox, unpublished results), but the purity of the preparations is insufficient to ascribe these activities to RecN with a high degree of confidence. The RecN protein exhibits a sequence relationship to the eukaryotic Smc proteins (134). This family of proteins generally has various roles in chromosome maintenance (23), such as chromosome condensation. These proteins have a predicted tertiary structure consisting of two domains connected by a hinge. Two parts of the ATPase active site are distributed between the two domains, such that the domains presumably must come together for ATP hydrolysis to occur (23, 134). Interestingly, the RecN protein is one of the most prominent proteins induced during the SOS response, expressed at levels similar to those of RecA protein (39, 68, 129, 146, 148, 167). Clearly, new approaches are needed to further investigate this interesting protein.

The RecQ protein is a DNA helicase (161, 162). Defects in certain mammalian homologues of RecQ helicase are associated with the human Werner's (180) and Bloom's (38) syndromes. In eukaryotes, the RecQ family of helicases appear to play roles in genome maintenance (17, 42, 143). The molecular role of RecQ helicase in the repair of replication forks has not yet been elucidated. Kowalczykowski and colleagues have demonstrated that RecQ will unwind covalently closed DNA circles in vitro and stimulate the strand passage activity of topoisomerase III. The end result is the catenation of the DNA circles (47). Given the topological constraints of many of the processes outlined in Figure 1 when they occur on the *E. coli* chromosome, such activities could play roles in several different processes in the repair of replication forks.

A major challenge in the way of a complete understanding of replication fork repair is the integration of these many protein functions, and probably more that remain undiscovered, into the various repair pathways.

THE POTENTIAL FOR ORIGIN-SPECIFIC RECOMBINATION-INDUCED REPLICATION DURING THE SOS RESPONSE

Many of the original clues that helped to elucidate the role of recombination in the repair of replication forks came from the study of replication restart during the SOS response (31), particularly the work of Kogoma (59). SOS is a complex physiological response, and the replication restart that occurs in it has both nonmutagenic and mutagenic components induced in separate stages (34, 68). The nonmutagenic responses that can be associated with the nonmutagenic restoration of replication forks are more important (58, 179).

Kogoma and coworkers found that DNA replication could be initiated without protein synthesis under conditions that induced the SOS response (60, 61), a phenomenon that was labeled stable DNA replication (SDR). Later, SDR associated with the SOS response was designated induced stable DNA replication or iSDR. This aspect of replication restart was also shown to be recombination dependent (6). The study of these phenomena provided much information about the proteins involved in the nonmutagenic repair of replication forks (31, 59), but some interesting mysteries remain. In particular, some of the many distinct recombination-dependent replication processes studied by Kogoma and coworkers were initiated largely at specific origins (59). These workers suggested that specific nucleases might be induced during the SOS response that would introduce site-specific double-strand breaks in the chromosome to initiate recombination. Such nucleases would be of considerable interest, and the origin-dependence of stable DNA replication during SOS deserves additional investigation.

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LITERATURE CITED

1. Al Deib A, Mahdi AA, Lloyd RG. 1996. Modulation of recombination and DNA repair by the RecG and PriA helicases of *Escherichia coli* K-12. *J. Bacteriol.* 178:6782–89
2. Anderson DG, Kowalczykowski SC. 1997. The recombination hot spot chi is a regulatory element that switches the polarity of DNA degradation by the RecBCD enzyme. *Genes Dev.* 11:571–81
3. Anderson DG, Kowalczykowski SC. 1997. The translocating RecBCD enzyme stimulates recombination by directing RecA protein onto ssDNA in a chi-regulated manner. *Cell* 90:77–86
4. Aravind L, Makarova KS, Koonin EV. 2000. Survey and summary: Holliday junction resolvases and related nucleases: identification of new families, phyletic distribution and evolutionary trajectories. *Nucleic Acids Res.* 28:3417–32
5. Arenson TA, Tsodikov OV, Cox MM. 1999. Quantitative analysis of the kinetics of end-dependent disassembly of RecA filaments from ssDNA. *J. Mol. Biol.* 288:391–401
6. Asai T, Bates DB, Kogoma T. 1994. DNA replication triggered by double-stranded breaks in *E. coli*: dependence on homologous recombination functions. *Cell* 78: 1051–61
7. Banerjee SK, Christensen RB, Lawrence CW, LeClerc JE. 1988. Frequency and spectrum of mutations produced by a

single cis-syn thymine-thymine cyclobutane dimer in a single-stranded vector. *Proc. Natl. Acad. Sci. USA* 85:8141–45
8. Barre FX, Soballe B, Michel B, Aroyo M, Robertson M, Sherratt DJ. 2001. Circles: the replication-recombination-chromosome segregation connection. *Proc. Natl. Acad. Sci. USA* 98:8189–95
9. Belguise-Valladier P, Maki H, Sekiguchi M, Fuchs RP. 1994. Effect of single DNA lesions on in vitro replication with DNA polymerase III holoenzyme. Comparison with other polymerases. *J. Mol. Biol.* 236:151–64
10. Bennett RJ, West SC. 1995. RuvC protein resolves Holliday junctions via cleavage of the continuous (noncrossover) strands. *Proc. Natl. Acad. Sci. USA* 92:5635–39
11. Bernstein H, Byerly HC, Hopf FA, Michod RE. 1985. Genetic damage, mutation, and the evolution of sex. *Science* 229:1277–81
12. Bernstein H, Hopf FA, Michod RE. 1988. Is meiotic recombination an adaptation for repairing DNA, producing genetic variation, or both? In *The Evolution of Sex: An Examination of Current Ideas*, ed. RE Michod, BR Levin, pp. 139–60. Sunderland, MA: Sinauer
13. Blanar MA, Sandler SJ, Armengod ME, Ream LW, Clark AJ. 1984. Molecular analysis of the recF gene of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 81:4622–26
14. Blattner FR, Plunkett GR, Bloch CA, Perna NT, Burland V, et al. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* 277:1453–74
15. Bosco G, Haber JE. 1998. Chromosome break-induced DNA replication leads to nonreciprocal translocations and telomere capture. *Genetics* 150:1037–47
16. Buhl SN, Stillman RM, Setlow RB, Regan JD. 1972. DNA chain elongation and joining in normal human and xeroderma pigmentosum cells after ultraviolet irradiation. *Biophys. J.* 12:1183–91
17. Chakraverty RK, Hickson ID. 1999. Defending genome integrity during DNA replication: a proposed role for RecQ family helicases. *BioEssays* 21:286–94
18. Chan SN, Vincent SD, Lloyd RG. 1998. Recognition and manipulation of branched DNA by the RusA Holliday junction resolvase of *Escherichia coli*. *Nucleic Acids Res.* 26:1560–66
19. Chiu SK, Low KB, Yuan A, Radding CM. 1997. Resolution of an early RecA-recombination intermediate by a junction-specific endonuclease. *Proc. Natl. Acad. Sci. USA* 94:6079–83
20. Churchill JJ, Anderson DG, Kowalczykowski SC. 1999. The RecBC enzyme loads RecA protein onto ssDNA asymmetrically and independently of chi, resulting in constitutive recombination activation. *Genes Dev.* 13:901–11
21. Clark AJ. 1974. Progress toward a metabolic interpretation of genetic recombination of *Escherichia coli* and bacteriophage lambda. *Genetics* 78:259–71
22. Clark AJ, Sandler SJ. 1994. Homologous genetic recombination: the pieces begin to fall into place. *Crit. Rev. Microbiol.* 20:125–42
23. Cobbe N, Heck MM. 2000. Review: SMCs in the world of chromosome biology—from prokaryotes to higher eukaryotes. *J. Struct. Biol.* 129:123–43
24. Cooper DL, Lahue RS, Modrich P. 1993. Methyl-directed mismatch repair is bidirectional. *J. Biol. Chem.* 268:11823–29
25. Cordeiro-Stone M, Makhov AM, Zaritskaya LS, Griffith JD. 1999. Analysis of DNA replication forks encountering a pyrimidine dimer in the template to the leading strand. *J. Mol. Biol.* 289:1207–18
26. Cox MM. 1993. Relating biochemistry to biology: how the recombinational repair function of the RecA system is manifested in its molecular properties. *BioEssays* 15:617–23
27. Deleted in proof
28. Cox MM. 1997. Recombinational crossroads—eukaryotic enzymes and the limits

of bacterial precedents. *Proc. Natl. Acad. Sci. USA* 94:11764–66
29. Cox MM. 1998. A broadening view of recombinational DNA repair in bacteria. *Genes Cells* 3:65–78
30. Cox MM. 1999. Recombinational DNA repair in bacteria and the RecA protein. *Prog. Nucleic Acids Res. Mol. Biol.* 63:310–66
31. Cox MM. 2001. Historical overview: searching for replication help in all the rec places. *Proc. Natl. Acad. Sci. USA* 98:8173–80
32. Cox MM, Goodman MF, Kreuzer KN, Sherratt DJ, Sandler SJ, Marians KJ. 2000. The importance of repairing stalled replication forks. *Nature* 404:37–41
33. Cromie GA, Leach DRF. 2000. Control of crossing over. *Mol. Cell* 6:815–26
34. Defais M, Devoret R. 2000. *SOS Response: Encyclopedia of Life Sciences* (online). Hampshire, UK: Macmillan
35. Diver WP, Sargentini NJ, Smith KC. 1982. A mutation (radA100) in *Escherichia coli* that selectively sensitizes cells grown in rich medium to x- or u.v.-radiation, or methyl methanesulphonate. *Int. J. Rad. Biol. Rel. Stud. Phys. Chem. Med.* 42:339–46
36. Dougherty EC. 1955. Comparative evolution and the origin of sexuality. *Syst. Zool.* 4:145–69, 90
37. Dutreix M, Rao BJ, Radding CM. 1991. The effects on strand exchange of 5′ versus 3′ ends of single-stranded DNA in RecA nucleoprotein filaments. *J. Mol. Biol.* 219:645–54
38. Ellis NA, Groden J, Te T-Z, Straughen J, Lennon DJ, et al. 1995. The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* 83:655–66
39. Finch PW, Chambers P, Emmerson PT. 1985. Identification of the *Escherichia coli* recN gene product as a major SOS protein. *J. Bacteriol.* 164:653–58
40. Flores MJ, Bierne H, Ehrlich SD, Michel B. 2001. Impairment of lagging strand synthesis triggers the formation of a RuvABC substrate at replication forks. *EMBO J.* 20:619–29
41. Flower AM, McHenry CS. 1991. Transcriptional organization of the *Escherichia coli* dnaX gene. *J. Mol. Biol.* 220:649–58
42. Frei C, Gasser SM. 2000. RecQ-like helicases: the DNA replication checkpoint connection. *J. Cell Sci.* 113:2641–46
43. Friedberg EC, Walker GC, Siede W. 1995. *DNA Repair and Mutagenesis*. Washington, DC: ASM Press. 698 pp.
44. Galitski T, Roth JR. 1997. Pathways for homologous recombination between chromosomal direct repeats in *Salmonella typhimurium*. *Genetics* 146:751–67
45. Gruber M, Wellinger RE, Sogo JM. 2000. Architecture of the replication fork stalled at the 3′ end of yeast ribosomal genes. *Mol. Cell. Biol.* 20:5777–87
46. Haber JE. 1999. DNA recombination: the replication connection. *Trends Biochem. Sci.* 24:271–75
47. Harmon FG, DiGate RJ, Kowalczykowski SC. 1999. RecQ helicase and topoisomerase III comprise a novel DNA strand passage function: a conserved mechanism for control of DNA recombination. *Mol. Cell* 3:611–20
48. Higgins NP, Kato K, Strauss B. 1976. A model for replication repair in mammalian cells. *J. Mol. Biol.* 101:417–25
49. Hill TM, Marians KJ. 1990. *Escherichia coli* Tus protein acts to arrest the progression of DNA replication forks in vitro. *Proc. Natl. Acad. Sci. USA* 87:2481–85
50. Horii Z, Clark AJ. 1973. Genetic analysis of the RecF pathway to genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. *J. Mol. Biol.* 80:327–44
51. Hyrien O. 2000. Mechanisms and consequences of replication fork arrest. *Biochimie* 82:5–17
52. Imlay JA, Linn S. 1986. Bimodal pattern of killing of DNA-repair-defective or anoxicallygrown *Escherichia coli* by hydrogen peroxide. *J. Bacteriol.* 166:519–27

53. Inman RB. 1984. Methodology for the study of the effect of drugs on development and DNA replication in *Drosophila melanogaster* embryonic tissue. *Biochim. Biophys. Acta* 783:205–15
54. Iype LE, Inman RB, Cox MM. 1995. Blocked RecA protein-mediated DNA strand exchange reactions are reversed by the RuvA and RuvB proteins. *J. Biol. Chem.* 270:19473–80
55. Jain SK, Cox MM, Inman RB. 1994. On the role of ATP hydrolysis in RecA protein-mediated DNA strand exchange III. Unidirectional branch migration and extensive hybrid DNA formation. *J. Biol. Chem.* 269:20653–61
56. Kaufmann WK, Cleaver JE. 1981. Mechanisms of inhibition of DNA replication by ultraviolet light in normal human and xeroderma pigmentosum fibroblasts. *J. Mol. Biol.* 149:171–87
57. Keyer K, Gort AS, Imlay JA. 1995. Superoxide and the production of oxidative DNA damage. *J. Bacteriol.* 177:6782–90
58. Khidhir MA, Casaregola S, Holland IB. 1985. Mechanism of transient inhibition of DNA synthesis in ultraviolet-irradiated *E. coli*: Inhibition is independent of *recA* whilst recovery requires RecA protein itself and an additional, inducible SOS function. *Mol. Gen. Genet.* 199:133–40
59. Kogoma T. 1997. Stable DNA replication: interplay between DNA replication, homologous recombination, and transcription. *Microbiol. Mol. Biol. Rev.* 61:212–38
60. Kogoma T, Lark KG. 1970. DNA replication in *Escherichia coli*: replication in absence of protein synthesis after replication inhibition. *J. Mol. Biol.* 52:143–64
61. Kogoma T, Lark KG. 1975. Characterization of the replication of *Escherichia coli* DNA in the absence of protein synthesis: stable DNA replication. *J. Mol. Biol.* 94:243–56
62. Kolodner R, Fishel RA, Howard M. 1985. Genetic recombination of bacterial plasmid DNA: effect of RecF pathway mutations on plasmid recombination in *Escherichia coli*. *J. Bacteriol.* 163:1060–66
63. Konforti BB, Davis RW. 1990. The preference for a 3′ homologous end is intrinsic to RecA-promoted strand exchange. *J. Biol. Chem.* 265:6916–20
64. Kowalczykowski SC. 2000. Initiation of genetic recombination and recombination-dependent replication. *Trends Biochem. Sci.* 25:156–65
65. Kowalczykowski SC, Dixon DA, Eggleston AK, Lauder SD, Rehrauer WM. 1994. Biochemistry of homologous recombination in *Escherichia coli*. *Microbiol. Rev.* 58:401–65
66. Kreuzer KN. 2000. Recombination-dependent DNA replication in phage T4. *Trends Biochem. Sci.* 25:165–73
67. Kuzminov A. 1996. *Recombinational Repair of DNA Damage*. Georgetown, TX: RG Landes. 210 pp.
68. Kuzminov A. 1999. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage lambda. *Microbiol. Mol. Biol. Rev.* 63:751–813
69. Kuzminov A. 2001. DNA replication meets genetic exchange: generation of chromosomal damage and its repair by homologous recombination. *Proc. Natl. Acad. Sci. USA* 98:8461–68

69a. Kuzminov A. 2001. Single-strand interruptions in replicating chromosomes cause double-strand breaks. *Proc. Natl. Acad. Sci. USA* 98:8241–46

70. Lawrence CW, Borden A, Banerjee SK, LeClerc JE. 1990. Mutation frequency and spectrum resulting from a single abasic site in a single-stranded vector. *Nucleic Acids Res.* 18:2153–57
71. Le S, Moore JK, Haber JE, Greider CW. 1999. RAD50 and RAD51 define two pathways that collaborate to maintain telomeres in the absence of telomerase. *Genetics* 152:143–52
72. Lehmann AR. 1972. Postreplication repair of DNA in ultraviolet-irradiated mammalian cells. *J. Mol. Biol.* 66:319–37

73. Lin PF, Howard-Flanders P. 1976. Genetic exchanges caused by ultraviolet photoproducts in phage lambda DNA molecules: the role of DNA replication. *Mol. Gen. Genet.* 146:107–15
74. Lindahl T. 1993. Instability and decay of the primary structure of DNA. *Nature* 362:709–15
75. Linn S, Imlay JA. 1987. Toxicity, mutagenesis and stress responses induced in *Escherichia coli* by hydrogen peroxide. *J. Cell Sci. Suppl.* 6:289–301
76. Liu J, Marians KJ. 1999. PriA-directed assembly of a primosome on D loop DNA. *J. Biol. Chem.* 274:25033–41
77. Livneh Z. 1986. Replication of UV-irradiated single-stranded DNA by DNA polymerase III holoenzyme of *Escherichia coli*: evidence for bypass of pyrimidine photodimers. *Proc. Natl. Acad. Sci. USA* 83:4599–603
78. Lloyd RG, Picksley SM, Prescott C. 1983. Inducible expression of a gene specific to the RecF pathway for recombination in *Escherichia coli* K12. *Mol. Gen. Genet.* 190:162–67
79. Lloyd RG, Sharples GJ. 1993. Dissociation of synthetic Holliday junctions by *E. coli* RecG protein. *EMBO J.* 12:17–22
80. Lovett ST, Clark AJ. 1984. Genetic analysis of the recJ gene of *Escherichia coli* K-12. *J. Bacteriol.* 157:190–96
81. Lovett ST, Kolodner RD. 1989. Identification and purification of a single-stranded-DNA-specific exonuclease encoded by the recJ gene of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 86:2627–31
82. Luder A, Mosig G. 1982. Two alternative mechanisms for initiation of DNA replication forks in bacteriophage T4: priming by RNA polymerase and by recombination. *Proc. Natl. Acad. Sci. USA* 79:1101–5
83. Mahdi AA, Lloyd RG. 1989. Identification of the recR locus of *Escherichia coli* K-12 and analysis of its role in recombination and DNA repair. *Mol. Gen. Genet.* 216:503–10
84. Mahdi AA, Lloyd RG. 1989. The recR locus of *Escherichia coli* K-12: molecular cloning, DNA sequencing and identification of the gene product. *Nucleic Acids Res.* 17:6781–94
85. Malkova A, Ivanov EL, Haber JE. 1996. Double-strand break repair in the absence of RAD51 in yeast: a possible role for break-induced DNA replication. *Proc. Natl. Acad. Sci. USA* 93:7131–36
86. Marians KJ. 1999. PriA: at the crossroads of DNA replication and recombination. *Prog. Nucleic Acids Res. Mol. Biol.* 63:39–67
87. Marians KJ. 2000. PriA-directed replication fork restart in *Escherichia coli*. *Trends Biochem. Sci.* 25:185–89
88. Maynard Smith J. 1978. *The Evolution of Sex*. Cambridge, UK: Cambridge Univ. Press
89. McEntee K, Weinstock GM, Lehman IR. 1980. RecA protein-catalyzed strand assimilation: stimulation by *Escherichia coli* single-stranded DNA-binding protein. *Proc. Natl. Acad. Sci. USA* 77:857–61
90. McGlynn P, Al DA, Liu J, Marians KJ, Lloyd RG. 1997. The DNA replication protein PriA and the recombination protein RecG bind D-loops. *J. Mol. Biol.* 270:212–21
91. McGlynn P, Lloyd RG. 1999. RecG helicase activity at three- and four-strand DNA structures. *Nucleic Acid Res.* 27:3049–56
92. McGlynn P, Lloyd RG. 2000. Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. *Cell* 101:35–45
93. McGlynn P, Lloyd RG, Marians KJ. 2001. Formation of Holliday junctions by regression of nascent DNA in intermediates containing stalled replication forks: RecG stimulates regression even when the DNA is negatively supercoiled. *Proc. Natl. Acad. Sci. USA* 98:8235–40
94. Meneghini R. 1976. Gaps in DNA synthesized by ultraviolet light-irradiated WI38

human cells. *Biochim. Biophys. Acta* 425:419–27
95. Meneghini R, Hanawalt P. 1976. T4-endonuclease V-sensitive sites in DNA from ultraviolet-irradiated human cells. *Biochim. Biophys. Acta* 425:428–37
96. Meselson M, Weigle JJ. 1961. Chromosome breakage accompanying genetic recombination in bacteriophage. *Proc. Natl. Acad. Sci. USA* 47:857–68
97. Michel B. 2000. Replication fork arrest and DNA recombination. *Trends Biochem. Sci.* 25:173–78
98. Michel B, Flores M-J, Viguera E, Grompone G, Seigneur M, Bidnenko V. 2001. Rescue of arrested replication forks by homologous recombination. *Proc. Natl. Acad. Sci. USA* 98:8181–88
99. Michel B, Recchia GD, Penel-Colin M, Ehrlich SD, Sherratt DJ. 2000. Resolution of Holliday junctions by RuvABC prevents dimer formation in rep mutants and UV-irradiated cells. *Mol. Microbiol.* 37:180–91
100. Michod RE. 1995. *Eros and Evolution: A Natural Philosophy of Sex*. Menlo Park, CA: Addison Wesley
101. Miguel AG, Tyrrell RM. 1986. Repair of near-ultraviolet (365 nm)-induced strand breaks in *Escherichia coli* DNA. The role of the polA and recA gene products. *Biophys. J.* 49:485–91
102. Modrich P. 1989. Methyl-directed DNA mismatch correction. *J. Biol. Chem.* 264: 6597–600
103. Moore PD, Bose KK, Rabkin SD, Strauss BS. 1981. Sites of termination of in vitro DNA synthesis on ultraviolet- and N-acetylaminofluorene-treated phi X174 templates by prokaryotic and eukaryotic DNA polymerases. *Proc. Natl. Acad. Sci. USA* 78:110–14
104. Morimyo M. 1982. Anaerobic incubation enhances the colony formation of a polA recB strain of *Escherichia coli* K-12. *J. Bacteriol.* 152:208–14
105. Morrical S, Hempstead K, Morrical M, Chou KM, Ando R, Grigorieva O. 1994. Mechanisms of assembly of the enzyme-ssDNA complexes required for recombination-dependent DNA synthesis and repair in bacteriophage T4. *Ann. NY Acad. Sci.* 726:349–50
106. Morrow DM, Connelly C, Hieter P. 1997. "Break copy" duplication: a model for chromosome fragment formation in *Saccharomyces cerevisiae*. *Genetics* 147:371–82
107. Morse LS, Pauling C. 1975. Induction of error-prone repair as a consequence of DNA ligase deficiency in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 72:4645–49
108. Mosig G. 1987. The essential role of recombination in phage T4 growth. *Annu. Rev. Genet.* 21:347–71
109. Mosig G. 1998. Recombination and recombination-dependent DNA replication in bacteriophage T4. *Annu. Rev. Genet.* 32:379–413
110. Nakayama H, Nakayama K, Nakayama R, Irino N, Nakayama Y, Hanawalt PC. 1984. Isolation and genetic characterization of a thymineless death-resistant mutant of *Escherichia coli* K12: identification of a new mutation (recQ1) that blocks the RecF recombination pathway. *Mol. Gen. Genet.* 195:474–80
111. Nilsen T, Baglioni C. 1979. Unusual base-pairing of newly synthesized DNA in HeLa cells. *J. Mol. Biol.* 133:319–38
112. Panyutin IG, Hsieh P. 1993. Formation of a single base mismatch impedes spontaneous DNA branch migration. *J. Mol. Biol.* 230:413–24
113. Panyutin IG, Hsieh P. 1994. The kinetics of spontaneous DNA branch migration. *Proc. Natl. Acad. Sci. USA* 91:2021–25
114. Papavinasasundaram KG, Colston MJ, Davis EO. 1998. Construction and complementation of a recA deletion mutant of *Mycobacterium smegmatis* reveals that the intein in *Mycobacterium tuberculosis*

recA does not affect RecA function. *Mol. Microbiol.* 30:525–34

115. Papavinasasundaram KG, Movahedzadeh F, Keer JT, Stoker NG, Colston MJ, Davis EO. 1997. Mycobacterial recA is cotranscribed with a potential regulatory gene called recX. *Mol. Microbiol.* 24:141–53
116. Park EM, Shigenaga MK, Degan P, Korn TS, Kitzler JW, et al. 1992. Assay of excised oxidative DNA lesions: isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. *Proc. Natl. Acad. Sci. USA* 89:3375–79
117. Parsons CA, Stasiak A, Bennett RJ, West SC. 1995. Structure of a multisubunit complex that promotes DNA branch migration. *Nature* 374:375–78
118. Pegg AE, Byers TL. 1992. Repair of DNA containing O6–alkylguanine. *FASEB J.* 6:2302–10
119. Perez-Roge RL, Garcia-Sogo M, Navarro-Avino J, Lopez-Acedo C, Macian F, Armengod ME. 1991. Positive and negative regulatory elements in the dnaA-dnaN-recF operon of *Escherichia coli*. *Biochimie* 73:329–34
120. Picksley SM, Attfield PV, Lloyd RG. 1984. Repair of DNA double-strand breaks in *Escherichia coli* K12 requires a functional recN product. *Mol. Gen. Genet.* 195:267–74
121. Postow L, Ullsperger C, Keller RW, Bustamante C, Vologodskii AV, Cozzarelli NR. 2001. Positive torsional strain causes the formation of a four-way junction at replication forks. *J. Biol. Chem.* 267: 2790–96
122. Potter H, Dressler D. 1988. Genetic recombination: molecular biology, biochemistry, and evolution. In *The Recombination of Genetic Material*, ed. KB Low, pp. 217–82. San Diego, CA: Academic
123. Rajagopalan M, Lu C, Woodgate R, O'Donnell M, Goodman MF, Echols H. 1992. Activity of the purified mutagenesis proteins UmuC, UmuD′, and RecA in replicative bypass of an abasic DNA lesion by DNA polymerase III. *Proc. Natl. Acad. Sci. USA* 89:10777–81
124. Rangarajan S, Woodgate R, Goodman MF. 1999. A phenotype for enigmatic DNA polymerase II: a pivotal role for pol II in replication restart in UV-irradiated *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 96:9224–29
125. Register JC III, Griffith J. 1985. The direction of RecA protein assembly onto single strand DNA is the same as the direction of strand assimilation during strand exchange. *J. Biol. Chem.* 260:12308–12
126. Richter C, Park JW, Ames BN. 1988. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc. Natl. Acad. Sci. USA* 85:6465–67
127. Robu ME, Inman RB, Cox MM. 2001. RecA protein promotes the regression of stalled replication forks in vitro. *Proc. Natl. Acad. Sci. USA* 98:8211–18
128. Roca AI, Cox MM. 1997. RecA protein: structure, function, and role in recombinational DNA repair. *Prog. Nucleic Acid Res. Mol. Biol.* 56:129–223
129. Rostas K, Morton SJ, Picksley SM, Lloyd RG. 1987. Nucleotide sequence and LexA regulation of the *Escherichia coli* recN gene. *Nucleic Acids Res.* 15:5041–49
130. Rothstein R, Michel B, Gangloff S. 2000. Replication fork pausing and recombination or "gimme a break". *Genes Dev.* 14:1–10
131. Rupp WD, Howard-Flanders P. 1968. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. *J. Mol. Biol.* 31:291–304
132. Rupp WD, Wilde CEd, Reno DL, Howard-Flanders P. 1971. Exchanges between DNA strands in ultraviolet-irradiated *Escherichia coli*. *J. Mol. Biol.* 61:25–44
133. Sagher D, Strauss B. 1983. Insertion of nucleotides opposite apurinic/apyrimidinic sites in deoxyribonucleic acid during in vitro synthesis: uniqueness

of adenine nucleotides. *Biochemistry* 22:4518–26
134. Saitoh N, Goldberg IG, Wood ER, Earnshaw WC. 1994. ScII: an abundant chromosome scaffold protein is a member of a family of putative ATPases with an unusual predicted tertiary structure. *J. Cell Biol.* 127:303–18
135. Sancar A, Sancar GB. 1988. DNA repair enzymes. *Annu. Rev. Biochem.* 57:29–67
136. Sandler SJ. 2000. Multiple genetic pathways for restarting DNA replication forks in *Escherichia coli* K-12. *Genetics* 155:487–97
137. Sandler SJ, Marians KJ. 2000. Role of PriA in replication fork reactivation in *Escherichia coli*. *J. Bacteriol.* 182:9–13
138. Sandler SJ, Samra HS, Clark AJ. 1996. Differential suppression of priA2::kan phenotypes in *Escherichia coli* K-12 by mutations in priA, lexA, and dnaC. *Genetics* 143:5–13
139. Saveson CJ, Lovett ST. 1999. Tandem repeat recombination induced by replication fork defects in *Escherichia coli* requires a novel factor, RadC. *Genetics* 152:5–13
140. Seigneur M, Bidnenko V, Ehrlich SD, Michel B. 1998. RuvAB acts at arrested replication forks. *Cell* 95:419–30
141. Seigneur M, Ehrlich SD, Michel B. 2000. RuvABC-dependent double-strand breaks in dnaBts mutants require RecA. *Mol. Microbiol.* 38:565–74
142. Shan Q, Bork JM, Webb BL, Inman RB, Cox MM. 1997. RecA protein filaments: end-dependent dissociation from ssDNA and stabilization by RecO and RecR proteins. *J. Mol. Biol.* 265:519–40
143. Shen JC, Loeb LA. 2000. The Werner syndrome gene: the molecular basis of RecQ helicase-deficiency diseases. *Trends Genet.* 16:213–20
144. Sherratt DJ, Arciszewska LK, Blakely G, Colloms S, Grant K, et al. 1995. Site-specific recombination and circular chromosome segregation. *Philos. Trans. R. Soc. London Ser. B: Biol. Sci.* 347:37–42
145. Shibata T, Das Gupta C, Cunningham RP, Radding CM. 1980. Homologous pairing in genetic recombination: formation of D loops by combined action of RecA protein and a helix-destabilizing protein. *Proc. Natl. Acad. Sci. USA* 77:2606–10
146. Shinagawa H. 1996. SOS response as an adaptive response to DNA damage in prokaryotes. *Exs* 77:221–35
147. Shlomai J, Polder L, Arai K, Kornberg A. 1981. Replication of phi X174 dna with purified enzymes. I. Conversion of viral DNA to a supercoiled, biologically active duplex. *J. Biol. Chem.* 256:5233–38
148. Smith BT, Walker GC. 1998. Mutagenesis and more: umuDC and the *Escherichia coli* SOS response. *Genetics* 148:1599–610
149. Smith GR. 1991. Conjugational recombination in *E. coli*: myths and mechanisms. *Cell* 64:19–27
150. Smith GR. 1998. DNA double-strand break repair and recombination in *E. coli*. In *DNA Damage and Repair, Vol 1: DNA Repair in Prokaryotes and Lower Eukaryotes,* ed. JA Nickoloff, MF Hoekstra, pp. 135–62. Totowa, NJ: Humana
151. Song Y, Sargentini NJ. 1996. *Escherichia coli* DNA repair genes radA and sms are the same gene. *J. Bacteriol.* 178:5045–48
152. Steiner WW, Kuempel PL. 1998. Sister chromatid exchange frequencies in *Escherichia coli* analyzed by recombination at the dif resolvase site. *J. Bacteriol.* 180:6269–75
153. Storz G, Imlay JA. 1999. Oxidative stress. *Curr. Opin. Microbiol.* 2:188–94
154. Svoboda DL, Vos JMH. 1995. Differential replication of a single, UV-induced lesion in the leading or lagging strand by a human cell extract—fork uncoupling or gap formation. *Proc. Natl. Acad. Sci. USA* 92:11975–79
155. Tang M, Bruck I, Eritja R, Turner J, Frank EG, et al. 1998. Biochemical basis of SOS-induced mutagenesis in *Escherichia coli*: reconstitution of in vitro lesion bypass dependent on the UmuD′2C

mutagenic complex and RecA protein. *Proc. Natl. Acad. Sci. USA* 95:9755–60
156. Tatsumi K, Strauss B. 1978. Production of DNA bifilarly substituted with bromodeoxyuridine in the first round of synthesis: branch migration during isolation of cellular DNA. *Nucleic Acids Res.* 5:331–47
157. Thomas DC, Veaute X, Fuchs RP, Kunkel TA. 1995. Frequency and fidelity of translesion synthesis of site-specific N-2-acetylaminofluorene adducts during DNA replication in a human cell extract. *J. Biol. Chem.* 270:21226–33
158. Thomas DC, Veaute X, Kunkel TA, Fuchs RP. 1994. Mutagenic replication in human cell extracts of DNA containing site-specific N-2-acetylaminofluorene adducts. *Proc. Natl. Acad. Sci. USA* 91:7752–56
159. Thompson BJ, Camien MN, Warner RC. 1976. Kinetics of branch migration in double-stranded DNA. *Proc. Natl. Acad. Sci. USA* 73:2299–303
160. Umezu K, Kolodner RD. 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.* 269:30005–13
161. Umezu K, Nakayama H. 1993. RecQ DNA helicase of *Escherichia coli*. Characterization of the helix-unwinding activity with emphasis on the effect of single-stranded DNA-binding protein. *J. Mol. Biol.* 230:1145–50
162. Umezu K, Nakayama K, Nakayama H. 1990. *Escherichia coli* RecQ protein is a DNA helicase. *Proc. Natl. Acad. Sci. USA* 87:5363–67
163. van Gool AJ, Hajibagheri NM, Stasiak A, West SC. 1999. Assembly of the *Escherichia coli* RuvABC resolvasome directs the orientation of Holliday junction resolution. *Genes Dev.* 13:1861–70
164. Vierling S, Weber T, Wohlleben W, Muth G. 2000. Transcriptional and mutational analyses of the *Streptomyces lividans* recX gene and its interference with RecA activity. *J. Bacteriol.* 182:4005–11
165. Viswanathan M, Lovett ST. 1998. Single-strand DNA-specific exonucleases in *Escherichia coli*. Roles in repair and mutation avoidance. *Genetics* 149:7–16
166. Voelkel-Meiman K, Roeder GS. 1990. Gene conversion tracts stimulated by HOT1-promoted transcription are long and continuous. *Genetics* 126:851–67
167. Walker GC, Smith BT, Sutton MD. 2000. The SOS response to DNA damage. In *Bacterial Stress Responses*, ed. G Storz, R HenggeAronis, pp. 131–44. Washington, DC: Am. Soc. Microbiol.
168. Wang TC, Chang HY, Hung JL. 1993. Cosuppression of recF, recR and recO mutations by mutant recA alleles in *Escherichia coli* cells. *Mutat. Res.* 294:157–66
169. Wang TC, Smith KC. 1986. Inviability of dam recA and dam recB cells of *Escherichia coli* is correlated with their inability to repair DNA double-strand breaks produced by mismatch repair. *J. Bacteriol.* 165:1023–25
170. Wanka F, Brouns RM, Aelen JM, Eygensteyn A, Eygensteyn J. 1977. The origin of nascent single-stranded DNA extracted from mammalian cells. *Nucleic Acids Res.* 4:2083–97
171. Webb BL, Cox MM, Inman RB. 1995. An interaction between the *Escherichia coli* RecF and RecR proteins dependent on ATP and double-stranded DNA. *J. Biol. Chem.* 270:31397–404
172. Webb BL, Cox MM, Inman RB. 1997. Recombinational DNA repair—the RecF and RecR proteins limit the extension of RecA filaments beyond single-strand DNA gaps. *Cell* 91:347–56
173. West SC. 1997. Processing of recombination intermediates by the RuvABC proteins. *Annu. Rev. Genet.* 31:213–44
174. West SC, Cassuto E, Howard-Flanders P. 1981. Heteroduplex formation by RecA protein: polarity of strand exchanges. *Proc. Natl. Acad. Sci. USA* 78:6149–53

175. Whitby MC, Ryder L, Lloyd RG. 1993. Reverse branch migration of Holliday junctions by RecG protein: a new mechanism for resolution of intermediates in recombination and DNA repair. *Cell* 75:341–50
176. Whitby MC, Vincent SD, Lloyd RG. 1994. Branch migration of Holliday junctions: identification of RecG protein as a junction specific DNA helicase. *EMBO J.* 13:5220–28
177. Wickner S, Hurwitz J. 1975. Association of phiX174 DNA-dependent ATPase activity with an *Escherichia coli* protein, replication factor Y, required for in vitro synthesis of phiX174 DNA. *Proc. Natl. Acad. Sci. USA* 72:3342–46
178. Witkin EM, Roegner MV. 1992. Overproduction of DnaE protein (alpha subunit of DNA polymerase III) restores viability in a conditionally inviable *Escherichia coli* strain deficient in DNA polymerase I. *J. Bacteriol.* 174:4166–68
179. Witkin EM, Roegner MV, Sweasy JB, McCall JO. 1987. Recovery from ultraviolet light-induced inhibition of DNA synthesis requires *umuDC* gene products in *recA718* mutant strains but not in *recA*$^+$ strains of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 84:6805–9
180. Yu C-E, Oshima J, Fu Y-H, Wijsman E, Hisama F, et al. 1996. Positional cloning of the Werner's syndrome gene. *Science* 272:258–62
181. Zannis-Hadjopoulos M, Persico M, Martin RG. 1981. The remarkable instability of replication loops provides a general method for the isolation of origins of DNA replication. *Cell* 27:155–63