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RecA-Dependent Recovery of Arrested DNA Replication Forks

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■ **Abstract** DNA damage encountered during the cellular process of chromosomal replication can disrupt the replication machinery and result in mutagenesis or lethality. The RecA protein of *Escherichia coli* is essential for survival in this situation: It maintains the integrity of the arrested replication fork and signals the upregulation of over 40 gene products, of which most are required to restore the genomic template and to facilitate the resumption of processive replication. Although RecA was originally discovered as a gene product that was required to change the genetic information during sexual cell cycles, over three decades of research have revealed that it is also the key enzyme required to maintain the genetic information when DNA damage is encountered during replication in asexual cell cycles. In this review, we examine the significant experimental approaches that have led to our current understanding of the RecA-mediated processes that restore replication following encounters with DNA damage.

CONTENTS

INTRODUCTION: REPLICATION RECOVERY IN UV-IRRADIATED BACTERIA: THE REQUIREMENT FOR RecA

All cells must faithfully replicate their entire genomic template in order to reproduce. Incomplete replication in any domain of the genome portends genomic instability, if not lethality, for the cell in which it occurs. Although normal cellular DNA replication is extremely processive, damage to the DNA template can prevent the replication machinery from accurately completing its task. DNA damage that arrests replication can result in deletions or genomic rearrangements if DNA synthesis resumes from the wrong sites, point mutations when an incorrect nucleotide is incorporated opposite a lesion in the template, or lethality when the block to replication cannot be overcome in some manner.

Replication forks encounter a variety of hurdles that might be expected to hinder their progression through the genome. These include endogenous lesions such as abasic sites from spontaneous depurination; oxidative lesions such as thymine glycol and 8-oxo-guanine; bulky chemical DNA adducts; photoproducts due to UV exposure; interstrand DNA crosslinks; single- and double-strand DNA breaks as a consequence of ionizing radiation; and possibly single-strand interruptions due to excision-repair events in progress. These biologically important lesions impose structurally diverse constraints on the DNA template and the translocating replication machinery. The recovery of replication in the face of these qualitatively different impediments must require different subsets of repair enzymes and diverse mechanistic pathways. For instance, thymine glycol is thought to block replication whereas 8-oxo-guanine does not (44, 197). However, 8-oxo-guanine frequently mispairs with adenine and can lead to mutagenesis when not repaired (19, 197). Both of these oxidative lesions are removed from the DNA template primarily through the action of glycosylases that specifically recognize these altered bases and remove them to initiate base excision repair (64, 177). Bulky base adducts, such as benzo(*a*)pyrene, also block the progression of the replication machinery but are repaired predominantly through the more general pathway of nucleotide excision repair (7, 72, 102, 161). In this case, the "abnormalities" in the DNA template are recognized and excised as part of a roughly 12-nucleotide segment, a repair patch is synthesized utilizing the intact strand as template, and the patch is then ligated to the contiguous parental DNA (148). DNA interstrand crosslinks such as those generated by *cis*-platin or photoactivated psoralens present a unique dilemma for the replication machinery, since the lesion prevents the unwinding of the duplex DNA and presumably prevents the polymerase from even reaching the lesion. Furthermore, because both strands of the DNA template are damaged, the lesion cannot be repaired simply by excision and resynthesis of the complementary strand, so

the repair process may include a recombinational event with an intact homologous duplex DNA. Although several models have been proposed (80, 115, 183), the mechanism of interstrand crosslink repair remains partially speculative. In the case of single-strand interruptions in the DNA template, it has been proposed that replication through these regions could lead to the formation of double-strand breaks and the collapse of the replication fork (91). In fact, in an early study it was suggested that such fork disruption might occur at the site of an excision-repair event in progress (59). However, it is still not known whether replication forks in *Escherichia coli* normally attempt translocation through regions that contain single- or double-strand breaks on the chromosome.

Considering the diversity of replication impediments and the corresponding repair pathways to deal with them, it becomes a daunting task to approach the general question of how the DNA template is accurately replicated in the presence of DNA damage. In order to understand how cells maintain genomic stability, it is important to understand how each of these impediments is processed. In this review, we focus upon the specific question of how replication forks respond and recover following encounters with the lesions produced by ultraviolet (UV) irradiation. It is hoped that an understanding of this situation will provide more general insights.

UV irradiation has served as a useful model to dissect many aspects of the general question of how replication recovers when it is blocked by DNA damage in one of the two parental strands. UV irradiation (at 254 nm) produces two primary DNA lesions, the *cis, syn*-cyclobutane pyrimidine dimer (CPD) and the more structurally distorting pyrimidine-6-4-pyrimidone photoproduct (6-4 PP) (121, 122). Both of these intrastrand pyrimidine dimers are formed in the DNA of bacteria (or other organisms) following exposure to sunlight. Although these lesions arrest progression of the DNA replication machinery (17, 156), growing cultures of *E. coli* survive after UV exposures that produce thousands of these lesions per genome (71), suggesting that there must be very efficient mechanism(s) to deal with the crisis when replication is arrested. It was observed over four decades ago that replication is transiently inhibited in *E. coli* following a moderate dose of UV irradiation, but that it efficiently resumes following a recovery period (63, 156, 157). In fact, photoreactivation (which specifically reverses the CPDs) was shown to shorten the recovery period (61). The *uvrA*, *uvrB*, and *uvrC* genes of *E. coli* are required to initiate nucleotide excision repair of UV-induced DNA lesions (reviewed in 148). *E. coli* strains mutated in any one of these genes are extremely sensitive to UV irradiation and unable to remove either CPDs or 6-4PPs from DNA. Furthermore, the recovery of DNA replication is severely impaired in these mutants (27, 71, 157). Therefore, one may conclude that the pathway of excision repair is essential for the efficient recovery of replication and survival of UV-irradiated *E. coli*.

The RecA protein is also central to the recovery of replication in UV-irradiated *E. coli*. Purified RecA cooperatively binds to single-strand DNA and then pairs it with homologous duplex DNA in vitro. The product of this reaction yields a RecA protein filament bound in a three-stranded DNA structure (for reviews see 86, 143, 165). RecA also binds to the single-strand regions generated at a replication

fork when it is impeded from progressing normally (142, 150). The bound RecA is "activated" and then serves as a transactivator for the upregulation of over 40 genes (31) that operate to maintain the integrity of the replication fork, repair the DNA lesions, carry out translesion DNA synthesis, and prevent premature cell division until the problems have been resolved (reviewed in 35, 49). This cellular response to replication arrest by exposure to UV irradiation, or many other agents that block replication, has been collectively termed the SOS response, after the international distress signal (138). The observation that non-growing cultures of *E. coli* do not induce a strong SOS response following UV irradiation supports the notion that active replication forks are critical for signaling the response (150). RecA bound to DNA functions as an activator through a two-component system that includes the LexA repressor (10, 93). LexA binds to a 20 base pair consensus sequence in the operator region of most of the DNA damage-inducible genes, suppressing their expression (11, 93, 189). Derepression of these genes occurs because RecA bound to single-strand DNA changes to a conformationally active state that promotes the autocatalytic cleavage of the LexA repressor (92). As the cellular concentration of LexA diminishes, the genes normally suppressed by LexA are more frequently transcribed.

Several of the genes upregulated by the SOS response are devoted to the task of clearing the lesions from the DNA template and restoring processive replication. Included among these are the gene products of *uvrA* and *uvrB* that are required for recognizing UV-induced lesions in the DNA template (48, 82). In fact, the upregulation of these genes is required for efficient global genomic repair of CPDs (36). A functional homolog of *uvrC*, *ydjQ* (now termed *cho*), is also upregulated but *uvrC* is not (45, 123). UvrC and Cho are enzymes that carry out the incision step for nucleotide excision repair. Other genes upregulated by RecA activation include *polB*, *dinB*, and *umuC* plus *umuD* that encode DNA polymerases Pol II, Pol IV, and Pol V, respectively. These alternative polymerases can facilitate replication through lesions that block the normal replication machinery (3, 8, 18, 73, 79, 81, 130, 137, 174, 188).

The act of RecA binding to the single-stranded DNA regions at blocked replication forks serves not only to "sense" that replication is blocked, but also to maintain the structural integrity of the replication fork itself until replication can resume (21, 71). It is well established that *recA* mutants are extremely hypersensitive to DNA damage. Following UV irradiation, *recA* mutants not only fail to recover replication but additionally exhibit a rapid degradation of the genomic DNA in a striking manner termed "*rec*-less" degradation (68, 151).

Thus, the simple binding of a complex protein, RecA, plays a critical role in directing the response when replication is in trouble. It serves both a structural role in protecting the replication fork DNA from degradation and as the trigger to upregulate genes that are responsible for restoring the DNA template and replication forks (Figure 1). It is the failure to accurately replicate the genomic template in the presence of DNA damage, whether spontaneous or induced, that is thought to produce most of the genetic instability and mutagenesis observed in cells of

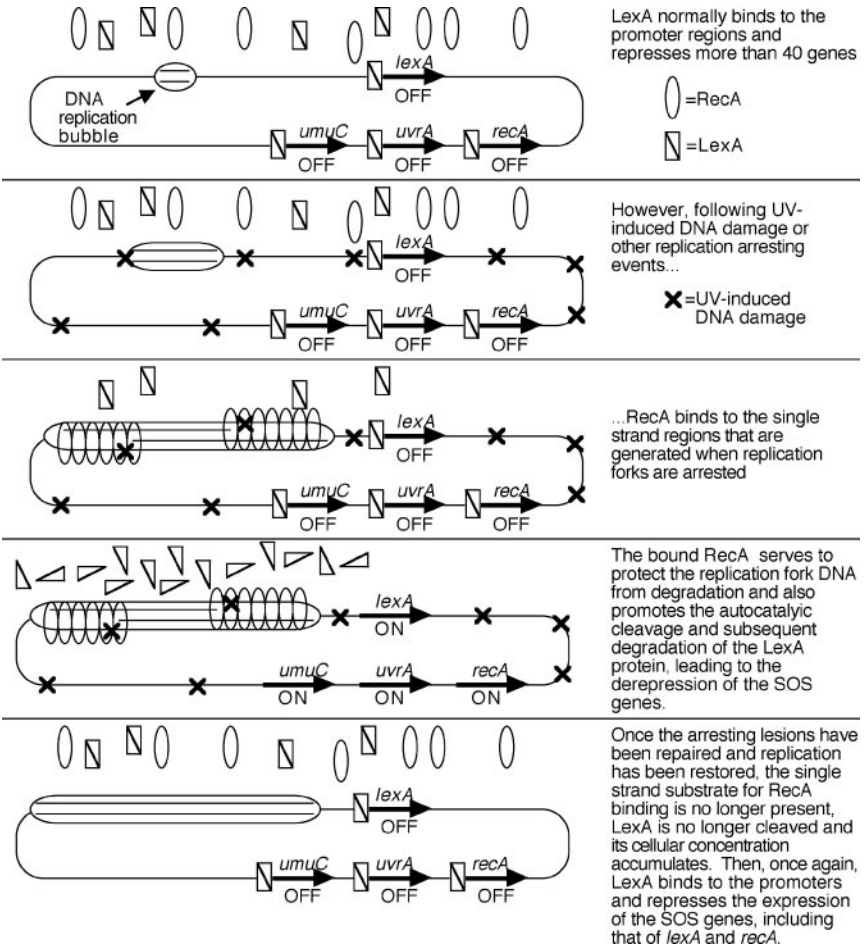

Figure 1 Regulation of SOS genes by RecA/LexA following UV-induced DNA damage.

all types. In addition to *recA*, a large number of candidate genes, when defective, also impair the accuracy and processivity of replication in the presence of DNA damage. Considering the severe consequences when replication goes awry, there is an intense interest to understand the molecular events that allow replication to accurately duplicate the genomic template when DNA damage is present. The functional mechanism of DNA replication is highly conserved among evolutionarily diverged organisms, making *E. coli* an extremely valuable and appropriate system for dissecting the molecular mechanisms by which replication recovers from DNA damage, as it is likely that these mechanisms will also extend to eukaryotes, including humans. In the following sections, we examine significant

experimental approaches that have led to our current understanding of the enzymatic activities and processing that can occur when replication forks encounter DNA damage.

MODELS AND CONCEPTS

Recovery by Recombination

A recent review states that "Replication forks frequently break and must be repaired by recombination" (98) and several recent opinions and perspectives have put forth this notion as well (32, 34, 89, 104, 113, 117). If one accepts that RecA is required for recombination in *E. coli* and if the above statement were rigorously true, then *recA* mutants should be inviable. While there is some lethal-sectoring in *recA* mutants, most of the bacteria survive, arguing that recombination is not generally essential for completing genomic replication in *E. coli*. This concept is discussed in more detail in the section on Enzymes with Potential Roles Following Replication Arrest.

Although RecA plays a critical role in the recovery of arrested replication forks, the *recA* gene was not initially discovered through studies directed to this question. Instead, *recA* was isolated in an attempt to determine the gene(s) responsible for the generation of genetic diversity during sexual cell cycles. In order to understand the mechanism by which genetic recombination occurs, Clark & Margulies utilized the sexual cycle of conjugation in *E. coli* to isolate mutants that could not undergo recombination during mating (21). Using this technique, they identified *recA* and, subsequently, several additional genes that are involved in the formation of recombinant molecules during bacterial sex. They observed that, in the absence of *recA*, no recombinant genomes were formed during conjugation, demonstrating that RecA was absolutely required for some step in the formation of recombinant DNA molecules. For many years following that seminal work, it has been assumed that the most important role of RecA is in genetic recombination.

In those initial studies, Clark & Margulies also made the critical observation that *recA* mutants, although viable, were extremely sensitive to UV light and to ionizing radiation during the normal asexual reproductive cycle. Howard-Flanders & Theriot in conjunction with Clark made the profound suggestion that recombination and DNA repair may involve common enzymatic steps (22, 70). Since recombination was the only activity known to be associated with *recA*, they proposed that RecA may also promote recombination during the asexual cell cycle as an alternative mechanism to the excision repair of DNA damage. A scheme termed postreplication repair was proposed, in which lesions in the template DNA were skipped over by the replication machinery to leave gaps in the product, and then those gaps were later closed through recombination with the partially replicated sequences from homologous undamaged regions (Figure 2). This process was later renamed daughter-strand gap repair to more precisely describe what was actually repaired, since, unlike excision repair, the process does not remove lesions from

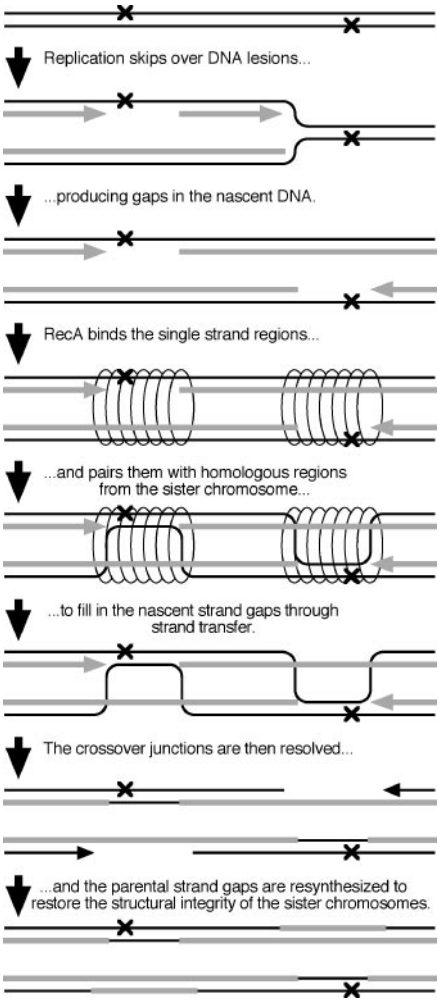

Figure 2 General model for postreplication recombination repair promoted by RecA.

the DNA (62). In that sense, this response should be considered a lesion-tolerance process rather than a repair pathway.

Additional experimental observations supported many features of this recombination model for overcoming replication blocks. It was shown that a mutation in *recA* further increased the sensitivity of a *uvrA* mutant to UV irradiation (Figure 3*A*) (71). It was concluded that since *uvrA* mutants are unable to remove UV-induced lesions from the genome, the limited survival of *uvrA* mutants following UV

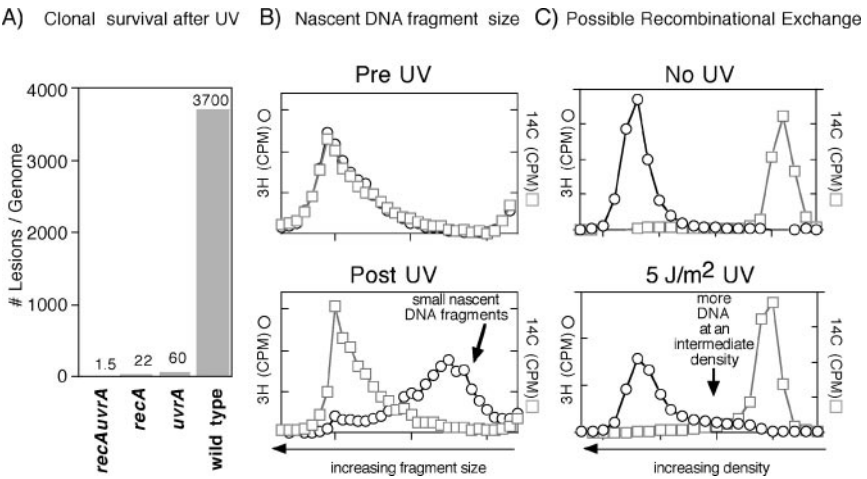

Figure 3 Experiments that have been interpreted to support the idea that *recA* promotes recombination repair during replication. *A.* The number of CPDs per genome that result in lethality for each bacterial strain is plotted, as calculated from (71). The extreme hypersensitivity of *recA* mutants led to the conclusion that recombination is important for repair. *B.* DNA labeled with $^{[3H]}$thymidine during the 2 minutes before or after UV irradiation in *uvrA* cultures is subsequently analyzed by alkaline sucrose gradient sedimentation to determine the relative fragment size of the nascent DNA. Following UV irradiation, the limited amount of observed DNA synthesis is in the form of short fragments, compared with that in unirradiated controls. This observation has been interpreted as replication skipping over DNA lesions and resuming downstream and as evidence that replication continues through DNA damage leaving gaps in the nascent DNA [data provided by C.T. Courcelle, essentially as described in (51)]. *C.* By transferring irradiated cells into media containing 5-bromouracil in place of thymine after irradiation, the postirradiation DNA synthesis can be separated from the DNA made before irradiation, based on differences in its buoyant density in CsCl gradients. One hour after irradiation, the DNA made by UV-irradiated *uvrA* cultures contains a higher proportion of intermediate density DNA than that from unirradiated control cultures. That observation has been interpreted to represent exchanges between parental and daughter DNA and as evidence that recombination repair is occurring [data provided by J.C., as described in (26)].

irradiation must be due to the ability of RecA to catalyze recombination following replication. It was also observed that when *recA* cultures were not replicating, they were more resistant to UV irradiation, again consistent with the idea that *recA* was required to "patch up" problems generated during replication.

Another observation supporting the recombination model was that the DNA synthesized immediately after UV irradiation in *uvrA* mutants consisted of short fragments that roughly corresponded in size to the average interlesion distance

(146). This was shown by separating the DNA fragments through zone sedimentation in alkaline sucrose gradients, and the results were consistent with the view that replication could skip over lesions in the template (Figure 3*B*), leaving gaps that were later filled.

A third observation suggested that, at later times, high frequencies of DNA strand exchanges were occurring in these UV-irradiated *uvr* mutants (147). By growing cells in different isotopic media before and after irradiation, the DNA made before and after irradiation could be separated in isopycnic CsCl gradients by differences in buoyant densities. To test whether recombination is induced by UV photoproducts, *uvr* mutants were irradiated with low-UV doses and allowed to recover for an hour. Compared with that in unirradiated *uvr* mutants, the irradiated cultures contained more DNA of an intermediate density, an observation that was interpreted to represent exchanges between parental and daughter DNA, and as evidence that recombinational "repair" was occurring (Figure 3*C*).

However, all of these observations were carried out in *uvr* mutant strains of *E. coli*. The rationale for doing this was that by eliminating the capacity for excision repair, the observed phenotypes could be entirely attributed to the proposed recombinational mechanism of repair. In addition, at the time these studies were conducted, the SOS response and the processes of translesion DNA synthesis were not known and so the concepts that upregulation of excision repair and translesion DNA synthesis may be involved in the RecA-dependent response were not considered. These and several subsequent studies are all consistent with the original model proposed in Figure 2. Due to the extreme hypersensitivity of *recA* mutants to DNA damage, the general view evolved that the proposed recombinational mechanism must represent a major repair pathway, required for cell survival and genomic stability.

However, this original recombination model for DNA repair did not account for several other experimental observations. Although RecA promoted limited survival in *uvrA* mutants, the survival of cells synergistically increased in the presence of excision repair (Figure 3*A*) (27, 29, 71). Fundamental genetics would argue that the contributions of truly independent pathways should be additive. Yet the UV survival of cells reduced to almost zero in the absence of either gene, suggesting that the major mechanism of recovery that operates in wild-type cells also involves excision repair. From a practical point of view, the survival curves suggested that if *recA* did promote DNA repair by recombination, it certainly did not operate with high efficiency in the absence of *uvrA*.

The recombination model also did not account for the observations that replication was strongly inhibited by UV-induced DNA lesions, that the inhibition was more severe, and that replication failed to significantly recover in either *recA* or *uvrA* mutants (27, 156). The recombinational model would otherwise imply that replication should continue at normal or near-normal rates through the lesion-containing parental DNA, leaving gaps at the lesion sites. Yet the actual amount of DNA synthesized in the form of short fragments following UV irradiation is estimated to represent synthesis extending only past one or two lesions before replication is inhibited (51–53).

According to the recombination-based models, one would predict that in the presence of DNA damage, the more strand exchanges that occur, the better off the cell should be. It was therefore difficult to explain the general observation that whenever significant levels of strand exchanges were detected in populations of cells (i.e., *uvr* mutants) there was a corresponding high level of cell death and mutagenesis. In the original study in which strand exchanges in irradiated *uvr* mutants were detected, no further growth was observed in the cell population (71, 146, 147). By contrast, in wild-type cells, which were able to recover and survive much higher levels of damage, the evidence for strand exchange or recombinational intermediates indicated that these events occurred at much lower frequencies (26, 27). This suggests that the recombination that was occurring might be detrimental to genomic stability, rather than a mechanism for maintaining it.

Following up on these early studies, a large amount of information about the biochemical and cellular functions of RecA has been obtained, but the fundamental idea that RecA always operates in a recombination mode to repair damage encountered by replication forks persists in the literature today. An important caveat must be added: We are discussing particular types of damage (i.e., photoproducts caused by UV) that only affect one DNA strand and are known to arrest the DNA replicase. Some types of damage affect both DNA strands within a spacing too short to accommodate excision repair. Thus, as noted earlier, processing of DNA lesions such as interstrand DNA crosslinkage or double-strand breaks may require both a recombinational event and some of the steps of excision repair. If a replication fork were to encounter a single-strand break in the duplex parental DNA (such as at a partially completed excision repair site) that would be expected to lead to a double-strand break—if the fork were allowed to pass through such a site (59).

Recovery Without Recombination, Maintaining the Arrested Replication Forks

In the nearly four decades since the recombination model was originally proposed, a large amount of information has accumulated about the complex phenotypes and activities associated with RecA, including its role in the upregulation of additional DNA polymerases and the excision repair genes, as well as the biochemical characterization of its strand-pairing and exchange activities. Additional genes have also been identified that are thought to have potential roles in the recovery of replication (for reviews, see 35, 86, 143, 165, 189). In order to incorporate these observations and to resolve some earlier discrepancies, the repair-by-recombination model has been revised to suggest that the strand-pairing activity of RecA is not required to rearrange the strands near replication forks, but rather to maintain the integrity of these arrested replication forks until they can resume DNA replication after the offending lesion has been removed (Figure 4) (26, 27, 29). A role in maintaining the integrity of blocked replication forks until repair can occur is more consistent with the observation that the survival promoted by *recA* synergistically increases when excision repair is also functional (Figure 3*A*) (71). This observation

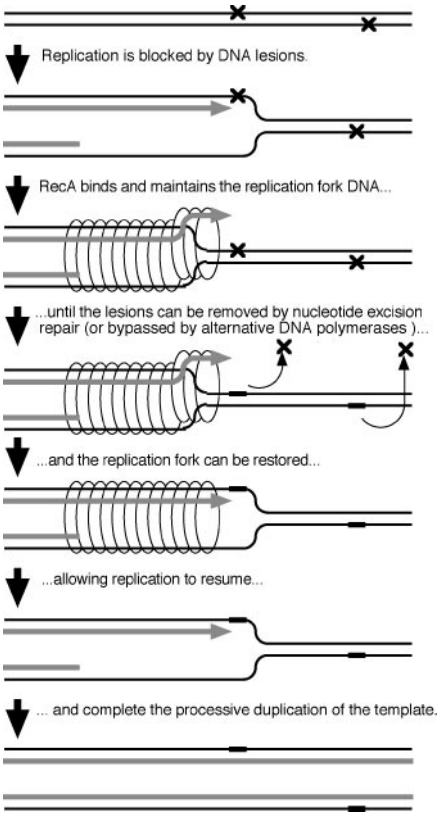

Figure 4 General model for the recovery of replication promoted by RecA.

indicates that a large number of the RecA-catalyzed events are also dependent upon the removal of the DNA lesions. This model also better explains the inhibition of replication that occurs after UV irradiation in all cell types, including the severe lack of recovery that occurs in either *recA* mutants or nucleotide excision repair mutants (Figure 5*A*) (71, 83, 157). Lastly, a model in which RecA simply maintains the fork until replication can resume is consistent with the observations that high levels of recombination are generally not observed nor beneficial to the asexual cell cycle.

The basic theme for this model was suggested by experiments carried out at approximately the same time that the recombinational repair model was proposed. Horii & Suzuki observed that following UV irradiation, *recA* mutants rapidly degrade most of their genomic DNA, a phenomenon termed *rec*-less degradation (Figure 5*B*) (68). This extensive DNA degradation did not occur in wild-type cells nor did it occur in *uvrA* mutants, despite the fact that *uvrA* mutants were similarly hypersensitive to UV. They also observed that the degradation was largely specific

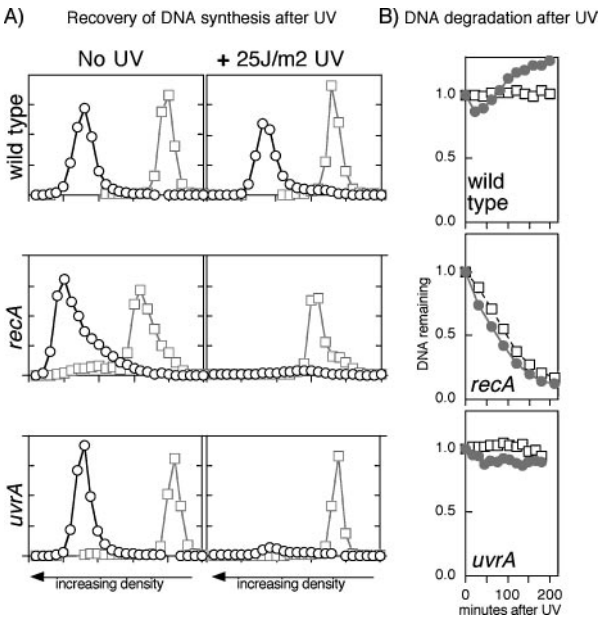

Figure 5 Experiments that have been interpreted to support the model for UvrA-dependent recovery of replication. *A*. Using the same assay described for Figure 3*C*, replication fails to recover in the absence of either *uvrA* or *recA* after a UV dose from which wild-type cells would completely recover. This observation has been interpreted to indicate that nucleotide excision repair is required for the RecA-promoted recovery of replication [data provided by J.C. as described in (26)]. *B*. The degradation of the nascent and bulk DNA that occurs following UV-irradiation is examined by adding $^{[3H]}$thymidine for 10 seconds to $^{[14C]}$thymine-prelabeled cells immediately before the cells are filtered and irradiated with 25 J/m2 UV in nonradioactive medium. The loss of bulk 14C DNA (*open squares*) can be compared with the loss of the nascent 3H DNA (*closed circles*). The bulk DNA in wild-type cells or in *uvrA* mutants remains protected following UV irradiation, and only limited degradation of the nascent DNA is detected. By contrast, *recA* mutants extensively degrade the bulk and nascent DNA following UV irradiation. That observation has been taken to indicate that RecA is required to maintain the integrity of replication forks following UV-induced DNA damage (data provided by J.C., as described in 30).

to bacterial cultures that were actively replicating DNA at the time the damage was incurred; based upon pulse labeling of nascent DNA, the degradation appeared to initiate at the replication forks themselves. These observations, in combination, led Horii & Suzuki to propose that RecA played a role in maintaining the integrity of replication forks that encounter DNA damage.

A second line of investigation, consistent with the idea that excision repair plays an important role in the RecA-promoted recovery process, comes from the

characterization of a phenomenon that was termed "long patch excision repair" (23, 24, 164). While characterizing the lengths of DNA that are excised and resynthesized at repair sites following DNA damage (often referred to as the "patch size"), it was observed that the size distribution of the excision repair patches in UV-irradiated *E. coli* was bimodal. Short patches appeared at early times and correlated with the now-known 10–12 nucleotide patch size of normal nucleotide excision repair. However, at times correlating with the robust recovery of replication, much longer "patches" of DNA synthesis were observed in a process dependent upon both *recA* and the nucleotide excision repair genes. These long patches were shown to localize primarily near DNA replication forks and were found to be either approximately 1500 bases or greater than 9000 bases in length. The long patch repair synthesis was also shown to be dependent upon SOS induction, as is the requirement to efficiently repair CPDs and resume replication (23). At the time, the authors concluded that these excision repair-dependent long patches were associated with the efficient recovery of replication (23). However, the size distribution of these "long patches" correlates well with those of the nascent DNA of lagging and leading strand synthesis, respectively, during normal replication. It is tempting to speculate that the long patches may represent the initial replication recovery following the removal of the blocking lesions by excision repair.

A model in which RecA functions to maintain the replication fork until the lesion can be repaired is consistent with several of the phenotypes associated with the recovery process. This model is also attractive when one characterizes several other gene products that function at the replication fork (see next section), although there are some shortcomings. If replication were always arrested by DNA lesions, that would suggest that no DNA synthesis should be detected following UV irradiation. However, as originally observed in the post-replication repair studies, short stretches of DNA synthesis equivalent to replication past approximately one or two lesions are observed at times immediately following UV irradiation (51, 53, 145, 146). Although most of these studies were carried out in *uvr* mutants, short nascent fragments can also be detected in wild-type cells at times immediately following UV irradiation (our unpublished observations). This suggests that although most UV-induced lesions arrest replication, not every lesion blocks replication. More recent studies, using defined substrates, suggest that this may indeed be the case and a possible explanation is offered to account for the limited synthesis that is observed (see section on The Arrested Replication Fork Substrate).

OTHER PROTEINS ACTING AT ARRESTED REPLICATION FORKS

In addition to RecA, several other gene products have been identified that operate at blocked replication forks following UV irradiation. These include the gene products in the RecF pathway, RecF, RecO, and RecR. Several lines of evidence, both in vivo and in vitro, suggest that they operate at a common step in promoting

RecA's ability to maintain the blocked replication fork structure, by stabilizing the activated form of the RecA filament bound to single-strand DNA. Mutants lacking any one, or all three, of these gene products are equally sensitive to DNA damage, although the sensitivity is not as great as that conferred by a mutation in *recA* (67, 85, 101). Mutations in *recF*, *recO*, or *recR* also delay the induction of gene expression regulated by LexA following DNA damage (65, 178, 194), consistent with the idea that there is less activated RecA present at early times if RecF-O-R proteins are absent. In vitro, RecF, RecO, and RecR can physically interact and, under some conditions, these proteins enhance the ability of RecA to bind and form filaments on single-strand DNA that is coated with single-strand binding protein (SSB) (180, 181, 192). In addition, these proteins have also been reported to limit the extension of RecA filaments into double-stranded DNA and to prevent RecA filaments from disassembling at DNA ends (9, 77, 159, 192, 193), as consistent with the general view that these proteins can enhance and stabilize RecA filaments in their activated and bound form. Also, strikingly similar to *recA* mutants, the *recF*, *recO*, and *recR* mutants severely fail to recover replication following UV-induced DNA damage (26, 140, 145).

Using a technique to differentially label the overall genomic DNA and the nascent DNA at the blocked replication forks in wild-type UV-irradiated cultures, it was observed that arrested replication forks normally undergo a limited amount of nascent DNA degradation at times prior to the recovery of replication (26, 60). However, when this same technique is applied to either *recF*, *recO*, or *recR* mutants, the nascent DNA degradation is much more extensive (K. H. Chow & J. Courcelle, unpublished results, 26), suggesting that these proteins are required along with RecA to maintain the integrity of the blocked replication fork. By contrast, although *uvrA* mutants also fail to recover replication, the nascent DNA remains protected and is not degraded more extensively than in wild-type cells (27). This observation is also consistent with the idea that RecF-O-R and RecA are able to protect and maintain the integrity of the replication fork. However, it suggests that when the blocking lesion cannot be removed, the replication machinery remains unable to efficiently resume synthesis of DNA.

Other proteins, associated with the RecF pathway, are involved in processing the replication fork before replication resumes. The nascent DNA degradation that occurs in wild-type cells and the more extensive degradation that occurs in *recF*, *recO*, or *recR* mutants have been shown to result from the combined action of the 3′–5′ helicase, RecQ, and the 5′–3′ single-strand exonuclease, RecJ (28, 30). Based upon the extent of nascent DNA degradation in *recF* mutants (30), the polarity of the helicase and nuclease in vitro (99, 182), and the preferential loss of nascent lagging-strand DNA at the fork (30), it was suggested that RecJ and RecQ specifically degrade the nascent lagging strand at replication forks when replication is blocked. In the absence of either gene product, the nascent DNA degradation does not occur, irrespective of whether RecF-O-R are present to maintain the replication fork (K. H. Chow & J. Courcelle, unpublished results, 28, 30). It is speculated that the RecJRecQ-mediated degradation generates a much more extensive substrate upon which RecA may bind and stabilize, thereby ensuring that replication resumes from

the same site at which disruption occurred. Consistent with this view, mutations in either *recQ* or *recJ* alter the frequency and sites at which recombination occurs on the chromosome (58, 162). By analogy, the *recQ* homologs in yeast, Drosophila, and humans play critical roles in maintaining processive replication and suppressing DNA strand exchanges (37, 42, 54, 78, 125, 153, 171, 190, 191, 199).

The *E. coli* genome also encodes three damage-inducible DNA polymerases, *polB* (Pol II), *dinB* (Pol IV), and *umuC* (Pol V), that are known to operate following DNA damage (3, 8, 18, 73, 79, 81, 130, 137, 174, 188). In vitro, these polymerases are able to incorporate nucleotides opposite specific DNA lesions with higher efficiency than the replicative polymerase, Pol III. Of course, the trade-off of utilizing a damaged template for replication is the potential for "misincorporation" and mutagenesis (3, 79). By placing either an N-2-acetylaminofluorene guanine (AAF), a benzo-a-pyrene (BaP), or a 6-4 PP lesion at a defined position on a plasmid template, it has been observed that the frequency of mutations and the mutation spectrum are different in *polB*, *dinB*, or *umuC* mutants, respectively, indicating that these polymerases are functioning at sites of DNA damage in some cases (50, 126, 187, 188). Furthermore, by pulse labeling with radioactive thymine, it has been shown that Pol II and Pol IV contribute to the postirradiation DNA synthesis that occurs during the recovery period (139, 140).

The complexity of the RecA participation in processes of replication recovery is abundantly illustrated by the example of translesion synthesis by Pol V. In addition to the requirement for RecA in the upregulation of the *umuC* and *umuD* genes, RecA also participates directly in the proteolytic cleavage of UmuD to its active form UmuD′ (131) and translesion synthesis by Pol V ($UmuD'_2$ UmuC) on the lesion-containing template (133, 141). During translesion synthesis, RecA interactions with Pol V both stimulate nucleotide incorporation and cocatalyze the bypass of the lesion in the DNA template (133). It is not presently known if the other translesion polymerases also require the presence of RecA for translesion synthesis.

However, the recovery of replication is not severely impaired in the absence of any or all of these inducible polymerases (J.C., unpublished observations). Furthermore, only *umuC, umuD* (Pol V) mutants render *E. coli* even moderately sensitive to UV-irradiation (R. Fuchs, personal communication; J.C., unpublished observations). This implies that these polymerases are not required to function at replication blocking lesions, although it does not preclude the likelihood that they can function at these sites in some cases. The modest effects upon survival and recovery may suggest that they function at alternative sites such as lesions that do not block the progression of the replication machinery, as discussed in the following section.

THE ARRESTED REPLICATION FORK SUBSTRATE

For clarity, diagrams of the replication machinery and the replication fork DNA are often drawn to comparable sizes. However, in the cell, the replication holoenzyme is a comparative giant relative to the DNA in the replication fork complex. Studies

of replication in vitro suggest that the polymerase generally will replicate all the way up to the nucleotide prior to the location of the blocking lesion (124). This raises the question, How does the repair machinery or a secondary DNA polymerase gain access to the blocking lesion when the replication machinery is sitting on top of it?

A potential strategy is suggested from the results of transcriptional studies. It is well established that a similar situation arises when an RNA polymerase encounters a blocking lesion in the transcribed strand (116, 154, 155). In this situation, the presence of the RNA polymerase prevents the excision repair machinery from gaining access to the lesion. Before repair can occur in *E. coli*, a special helicase-like protein (but it does not operate as a helicase), encoded by *mfd*, is needed to displace the RNA polymerase and the nascent RNA transcript. In *mfd* mutants, transcription-blocking lesions are not rapidly repaired and the cells are moderately UV sensitive (116). By analogy, one could expect that repair of a replication blocking DNA lesion might also require that the DNA polymerase and nascent DNA be transiently displaced. Using two-dimensional agarose gel analysis to examine replicating plasmids in *E. coli*, a transient reversal of the replication fork is observed following UV irradiation (28). The regressed fork structure is not maintained in *recF*, *recO*, or *recR* mutants, although it is partially restored when either *recJ* or *recQ* is also inactivated. Many of the regressed substrates are less than fully replicated in length. However, since RecQRecJ are thought to target the nascent lagging strand, it can be inferred that the regressed intermediate may often contain a lagging-strand extension. Also of interest to note from this study is that the intermediate persists for a period that correlates with that for the repair of lesions and the resumption of replication. It remains to be determined whether such a structure also occurs on the chromosome in vivo. However, a similar structure has been observed on the chromosome in yeast following hydroxyurea treatment (166). Hydroxyurea is thought to stall the DNA replication machinery by reducing the available pools of nucleotide precursors (134, 200).

A combination of in vitro and in vivo approaches has helped to define the substrates produced when the replication fork encounters a DNA lesion in the template. Interestingly, but not surprisingly, the situation appears to depend on which strand contains the DNA lesion. Following reconstitution of the replication holoenzyme on a plasmid substrate, a block to the leading-strand polymerase was found to arrest the entire replication machinery (P. McInerney & M. O'Donnell, personal communication). The replication fork arrested with the leading-strand synthesis at the block site. However, lagging-strand synthesis continued on beyond the block site before arrest occurred. The replication arrest was observed when the polymerase was blocked either by the incorporation of a dideoxynucleotide in the nascent DNA or by an abasic site in template of the leading strand. By contrast, when the block to the polymerase was placed in the lagging-strand template, no significant disruption in the rate of incorporation was observed (P. McInerney & M. O'Donnell, personal communication). Similar observations are seen in vivo, by examining the replication products produced when a plasmid that contains a

specific AAF adduct in either the leading- or the lagging-strand template (50, 132). In both cases, DNA synthesis on the strand without a lesion continued past the site of the lesion on the other strand.

These studies indicate that the replication fork arrests when a block to the leading strand polymerase is encountered, but can continue when a block to the lagging strand polymerase is encountered. Studies using SV40 plasmids in mammalian systems have suggested that the eukaryotic replication machinery behaves similarly (25, 172, 173, 185, 186). Based upon our understanding of the dynamic structure of the replication holoenzyme coordination of leading- and lagging-strand synthesis, it seems reasonable to suggest that the leading-strand block arrests replication because there is no mechanism to allow priming and resumption of replication downstream of the lesion. By contrast, the lagging-strand polymerase must periodically release and reinitiate synthesis as new primers arise on the lagging-strand template. This implies that when the lagging-strand polymerase is blocked before completing an Okazaki fragment, it may simply release and reinitiate from the next downstream primer, leaving a gap in the nascent lagging strand (potentially a prime substrate for a translesion DNA polymerase).

These observations also provide a potential explanation for the limited amount of nascent DNA that is produced following UV irradiation. Assuming that the lesions are randomly distributed in the leading and lagging strands, half of the replication forks will encounter a lesion in the leading strand first, whereas the other half of the replication forks will pass over one or more lesions in the lagging strand prior to replication arrest. Thus, based upon our current understanding, in a population of UV-irradiated *E. coli* cells, it is believed that replication forks arrest with the leading strand blocked at the lesion and the nascent lagging strand extending some distance ahead of it. In addition, the nascent lagging strand may contain one to a few gaps, depending upon how many lesions in the lagging-strand template have been encountered before a lesion is encountered in the leading strand template (Figure 6).

OTHER IMPEDIMENTS TO REPLICATION AND THE FREQUENCY OF DISRUPTION

RecA is thought to be required for cell survival whenever replication is disrupted. This concept was inferred from the original studies of Howard-Flanders and colleagues with UV-irradiated *E. coli*. They noted that if the DNA lesions cannot be repaired (e.g., in a *uvrA* mutant), then a UV dose that produced only 1–2 lesions per genome was sufficient to inactivate *recA* mutants (71). This implied that in the absence of RecA, replication disruption would be lethal. Surprisingly, in the absence of any exogenous DNA damage, *recA* mutants grow remarkably well (Figure 7*B*) (13–16, 119, 170). Viability is reduced approximately 20%, although the extent to which growth is impaired varies depending on strains and growth conditions. This suggests to us that the baseline frequency at which replication is

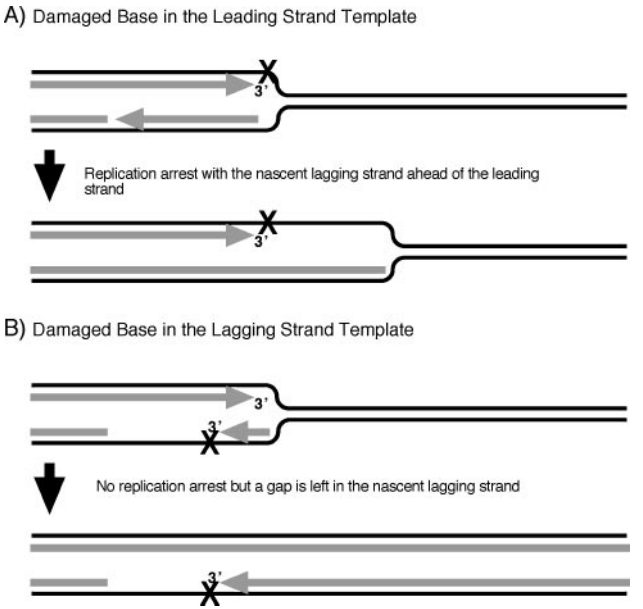

Figure 6 Likely substrates generated following replication encounters with DNA lesions in the leading-strand template or lagging-strand template.

normally disrupted in the absence of externally imposed DNA damage must be relatively low. It must be significantly less than 50% of the time each generation, if *recA* mutants are to survive and form colonies.

It then becomes interesting to consider that replication forks must encounter a wide variety of hurdles that may hinder their progression through the genome. These may include unusual secondary structures in the DNA, proteins bound to the DNA, or even translocating RNA polymerases. Clearly, the viability of *recA* mutants would argue that either these events do not normally disrupt replication or that they do not require RecA-mediated restoration. Rather, it suggests that the replication apparatus is comparatively stable and may react to many of these impediments by pausing, slowing down, or stalling. In vitro, Pol III holoenzyme remains stably bound to the DNA for more than 20 minutes when nucleotides are depleted (P. McInerney & M. O'Donnell, personal communication). Similarly, *recA* cultures are able to resume replication normally following transient resuspensions in buffers that stall replication due to a lack of nucleotide precursors, suggesting that pausing without disruption is an option for the replication machinery (26, 27).

This idea contrasts with the prevalent view that replication is frequently disrupted during genomic duplication and must be repaired through recombination [for some recent reviews expressing this viewpoint see (32, 34, 89, 98, 104, 113, 117)]. This viewpoint arose as a means to explain the poor growth and low

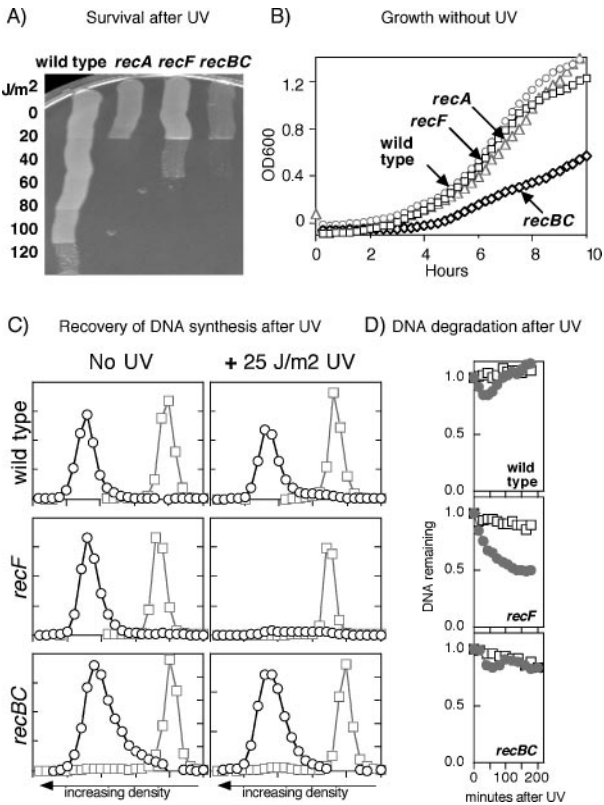

Figure 7 The phenotypes displayed by *recF* and *recBC* mutants, emphasizing the need to characterize UV-sensitive and growth-impaired mutants in vivo. *A*. The UV sensitivity of wild-type, *recA*, *recF*, and *recBC* mutants is shown. *recF* mutants and *recBC* mutants are equally sensitive to UV. *B*. The growth of *recA*, *recF*, and *recBC* mutants is plotted over time as measured by the relative optical density of the culture. In the absence of DNA damage, the growth and viability of *recBC* mutants is impaired relative to that of *recA* or *recF* mutants. *C*. Recovery of replication determined directly by using the assay described in Figure 3*C*: the *recBC* mutants recover replication normally. By contrast, *recF* mutants are severely impaired in their ability to resume replication following DNA damage, yet they grow normally in the absence of DNA damage. *D*. By examining the degradation of the nascent and bulk DNA following UV-irradiation as described in Figure 5*B*, the extensive degradation of the nascent DNA in *recF* mutants also implicates this gene in the recovery of replication. By contrast, the nascent and bulk DNA remain protected in *recBC* mutants and in wild-type cells. Bulk ^{14}C DNA (*open squares*); nascent ^{3}H DNA (*closed circles*). (Data provided by J.C.)

viability of mutants that are also associated with impaired recombination proficiency, such as *recBC* mutants, *priA* mutants, or *ruvABrecG* triple mutants, among others. In most cases, the impaired growth and viability of these mutants is much more severe than that of *recA* (Figure 7*B*). However, virtually all of the proposed recovery models are absolutely dependant upon RecA for either recombination or the re-establishment of a replication fork. Then, the comparative health and viability of *recA* mutants in relation to these other mutants argues against this interpretation. Instead, the viability problems of these mutants may suggest that they are required for additional roles during the cell cycle other than the recombination process by which they were originally identified. This, of course, does not preclude the possibility that the gene products also participate in the re-establishment of replication following disruption. Alternatively, these gene products could contribute to the stability of replication machinery itself such that disruption occurs more frequently in their absence. Importantly, however, many of the genes that have been proposed to be involved in the recovery of blocked replication forks have yet to be examined directly. The operation of the replication fork is but one of a large number of critical activities that are required for cellular reproduction. An important approach would be to examine precisely where and when the defects in each of these mutants are manifested during the normal reproductive cycle.

In the absence of exogenous DNA damage, it is estimated that the need for RecA-mediated recombination is extremely low. Using an analysis based upon chromosome linkage during replication, Steiner & Kuempel estimated the frequency of sister chromatid exchange in *E. coli* based on the frequency that replication generated circular dimer chromosomes rather than monomers (170). Using 5-bromodeoxyuridine to density label and isolate the chromosomes, they estimated that an exchange event occurs in only 15 percent of *E. coli* replication cycles. The actual value is likely to be much lower considering that 5-bromodeoxyuridine is also known to stimulate sister chromatid exchanges in bacterial cells. The toxicity of 5-bromodeoxyuridine is thought to be due in part because the analog is not incorporated with the same efficiency as the natural thymine by the replication machinery (57) and also because the bromine group on the analog is somewhat labile and this leads to the initiation of uracil glycolyase-induced nicks in the DNA (87). This results in total failure of replication and cell death within approximately two rounds of replication. Thus, the cell cultures in which these exchange events were measured is not destined for high viability.

ENZYMES WITH POTENTIAL ROLES FOLLOWING REPLICATION ARREST

RecBCD

RecB, RecC, and RecD form a helicase/nuclease complex that is required for the repair of double-strand DNA breaks in *E. coli*. *recB* and *recC* mutants were originally isolated based upon their severely impaired ability to form recombinant

molecules during conjugation and transduction (43). In addition, however, *recB* and *recC* mutants exhibit very low viability (much lower than that of a *recA* mutant!), and they are hypersensitive to forms of DNA damage that do not directly produce double-strand breaks (16, 43). The RecBCD enzyme complex can bind, translocate, and unwind DNA from a double-strand DNA end (144, 175). The unwinding is associated with a nuclease activity that preferentially degrades the 3′-ended strand until it encounters a Chi sequence, 5′GCTGGTGG3′, at which point the strand specificity of degradation switches to the 5′-ended strand (39, 135, 176). The Chi sequences and a 5-10-kb region upstream of the Chi sequence are hotspots for recombinational crossovers (167–169). In vitro, the processing of DNA ends by RecBCD promotes loading of RecA filaments and joint molecule formation, and it is postulated that this function also serves to initiate recombination and strand exchange in vivo (1, 144). However, it remains to be determined whether RecBCD also participates in the later steps of double-strand break repair or whether it is additionally active upon other substrates in vivo.

As a possible way to explain the UV-sensitive phenotype and poor viability of *recBC* mutants, it was speculated that replication forks may frequently "collapse" to form double-strand breaks at replication-blocking impediments (6, 90, 91). If true, then it was predicted that RecBCD would be required to process the products of the collapse in order to allow replication to recover. In support of this model, using pulse field gel electrophoresis to examine the overall integrity of the genomic DNA, double-strand breaks were seen to accumulate in the genome of certain thermosensitive DNA replication mutants following extended incubation at the nonpermissive temperature (118). Using this type of assay for double-strand breaks as a model "lesion" that impairs replication progression, elevated levels of double-strand breaks are detected in the *E. coli* genome for a number of replication impaired mutants, including *rep*, *dnaBts*, *dnaEts*, *dnaNts*, *holDts*, and *dnaGts* (46, 47, 56, 118). The level of double-strand breaks is higher if the cells additionally are deficient in *recBrecC*, indicating that RecBCD plays a role in the prevention or repair of double-strand breaks that arise under these conditions.

Based upon these observations, it was speculated that the loss of certain replication proteins may mimic the replication disruption caused by some DNA lesions and that this event may require RecBCD to enable the replication forks to resume (118). However, when this possibility was tested directly in UV-irradiated *E. coli*, the absence of RecBC or RecD did not affect the nascent DNA processing at arrested replication forks (30). In addition, unlike *recF*, *-O*, or *-R* mutants, *recBC* and *recD* mutants recovered replication normally following UV-induced DNA damage (26, 30, 83). Furthermore, when *dnaBts* mutants are shifted to the restrictive temperature, the nascent DNA at the replication forks is degraded independently of RecD function. The nascent DNA degradation in this case is also independent of RecJ and RecQ function, unlike following UV irradiation, and it occurs even when RecF is functional (J.J. Belle & J. Courcelle, unpublished observations). Lastly, double-strand breaks do not accumulate in the genome in *recF* mutants as they do in *recBC* mutants (118). Taken together, these observations indicate that the events

and enzymes operating at DNA lesion-blocked replication forks are different from those that occur in the absence of certain replication proteins.

Other differences between these various forms of replication inhibition also should be taken into account when estimating their biological significance. In the case of UV irradiation, *E. coli* cultures are capable of surviving events that produce thousands of lesions per genome, implying that there is a very efficient mechanism operating in the cell to allow replication to deal with these events successfully. By contrast, cells lacking replication proteins are not in the process of recovering. Although clearly revealing interesting enzymology, it is important to consider that the missing replication proteins are undoubtedly required in the normal recovery process. It then seems reasonable to infer that the ensuing enzymology may be revealing events that take place when recovery cannot occur. Furthermore, it has not yet been determined where or how the double-strand breaks arise in the genome. Are they even at the sites of replication forks? It remains an interesting question as to when and upon which substrates RecBCD acts during both the normal reproductive cycle and following DNA damage. The poor viability in the absence of DNA damage and the UV hypersensitivity of *recBC* mutants indicate that this enzyme complex plays an important role in promoting cell survival. However, as the recovery of replication following UV irradiation demonstrates, the function of this enzyme complex is not required for arrested replication forks to resume (26).

Other curious observations associated with the RecBCD enzyme include its role in suppressing lytic replication in phage lambda and phage P22 (136), its suppression of rolling circle replication on plasmids (88, 163), and its predominance to act in the terminal region of the chromosome (69). It is also interesting that, for as-yet undefined reasons, Chi sequences are predominantly oriented in the direction of the progressing replication forks and that predominance is more heavily biased closer to the chromosomal origin of replication (12; J.C., unpublished observations). It will be important to incorporate these observations as the cellular roles of this multifaceted enzyme continue to be characterized.

RuvABC

RuvA, RuvB, and RuvC form an enzymatic complex that functions as a "resolvasome" that is active on Holliday junctions, which form during the processes of recombination and replication. In vitro, RuvA, a junction-specific binding protein, and RuvB, which forms a hexameric ring, interact and cataylze branch migration on three-arm and four-arm (Holliday) junctions (66, 74, 179). RuvC interacts with RuvA and B and encodes an endonuclease that is specific for Holliday junctions, producing symmetric cuts at the point of crossover to resolve joint molecules (5, 75, 158). Mutants lacking any one or all three of these genes are moderately sensitive to DNA damage and also exhibit reduced recombination frequencies during conjugation or transduction (96, 160). Due to the structural similarities between three- and four-arm Holliday junctions and replication forks, it has been proposed that RuvABC may function in vivo to catalyze the reversal of replication forks

blocked at DNA lesions (112, 152). Such reactions have been shown to occur on synthetic replication fork structures in vitro (112).

In vivo, *ruvAB* or *ruvC* mutants recover replication at times similar to those for wild-type cells following UV-induced DNA damage (J. R. Donaldson & J. Courcelle, unpublished results). In addition, the absence of these genes does not result in extensive degradation of the replication fork DNA, nor does it prevent the degradation from occurring if RecF, -O, or -R proteins are not present (J. R. Donaldson & J. Courcelle, unpublished results). These observations suggest that if RuvABC does catalyze replication fork regression, then the regression is not required for the normal resumption of DNA synthesis or processing of the nascent DNA at the replication fork. Curiously, when replication is inhibited through the loss of thermosensitive replication proteins (e.g., DnaB), the formation of double-strand breaks depends on the RuvABC function, suggesting that double-strand breaks that arise in this situation are formed through the RuvABC cleavage of Holliday junctions (152). Also noteworthy is the observation that following UV irradiation of *ruvAB* mutants, although plasmid replication resumes normally in vivo, the synthesis produces large multimeric recombination structures (J.R. Donaldson & J. Courcelle, unpublished observations), indicating that these genes participate in the resolution of Holliday structures that form in some stage of the recovery process following DNA damage. Precisely when and upon which substrates this enzyme complex acts in vivo remain to be characterized.

RecG

Similar to RuvAB, the RecG helicase also catalyzes branch migration on forked DNA structures, and *recG* mutants confer a modest sensitivity to DNA damage and a moderate reduction in recombination frequency (103, 195). Using various synthetic substrates, purified RecG has been suggested to cataylze branch migration on 3- or 4-stranded junctions in a manner that can promote or abort strand exchange (195). It is also capable of promoting the displacement of the leading strand of the replication fork and the lagging strand of the replication fork (109, 114, 111). Based primarily on survival curves of *recG* mutants in various genetic backgrounds, RecG has been widely speculated to participate in a number of replicational recovery pathways (38, 55, 110, 113, 111). The idea that RecG may be required to displace arrested replication machinery and nascent DNA has also been suggested, based upon analogies to transcriptional encounters with DNA damage (29, 154). This concept is especially tempting considering that *recG* shares regions of homology with *mfd* (2, 97), the helicase-like protein that is required to displace the RNA polymerase and transcript prior to repairing lesions at blocked sites of transcription (154).

However, following UV-induced DNA damage, *recG* mutants recover replication with kinetics similar to those of wild-type cells (J. R. Donaldson & J. Courcelle, unpublished results). Similar to the case with *ruvAB*, however, *recG* is not required to maintain the integrity of the replication fork, nor does it affect the nascent DNA degradation that normally occurs prior to recovery

(30; J. R. Donaldson & J. Courcelle, unpublished results). Although these assays do not preclude a role for RecG at arrested replication forks, they do indicate that RecG is not essential for resumption of replication following disruption by UV-induced DNA damage. Further characterization of the in vivo substrates and functions for RecG is necessary before a role for this gene product can be determined with confidence.

PriA

PriA is a factor required for primosome assembly during the initiation of replication on the single-strand phage ØX174 in vitro (196). It functions together with PriB, PriC, and DnaT in a reaction that promotes the loading of the replication helicase, DnaB, and then primase, DnaG. During chromosomal replication, the primasome (DnaB and DnaG) tracks along the lagging-strand template and serves to unwind and repeatedly prime the template DNA for lagging-strand DNA synthesis (reviewed in 105). In vitro, PriA and the other primasomal assembly proteins are not required to initiate replication on *oriC* plasmids. However, they are required for replication on other templates, including ColE1 plasmids, suggesting that PriA is essential for replication in some cases (120). Furthermore, several phenotypes associated with *priA* mutants suggest that PriA contributes to the stability or processivity of the replication machinery in vivo as it progresses through the genome. Mutants lacking PriA exhibit low viability, slow growth, and chronic SOS induction, the latter result being indicative of an abnormal replication fork structure (107, 149, 196). In addition, *priA* mutants are impaired for replication-mediated forms of recombination and are hypersensitive to DNA-damaging agents (95, 107, 108, 149, 196). The poor growth and low viability of *priA* mutants are often taken to indicate that replication forks are frequently disrupted even in the absence of exogenous DNA damage and require genes such as *priA* for restoration of the replication fork (32, 33, 89, 98, 100). Consistent with this view, elegant biochemical studies have shown that PriA can participate in a reaction that facilitates loading of the DnaB helicase at replication fork structures in vitro, in a reaction similar to that thought to occur at the origin of replication on the *E. coli* chromosome (76, 94, 104, 106, 127–129, 198). However, as mentioned above, all models for the recovery of disrupted replication forks also require the pairing activities of RecA for strand invasion. Thus, the comparative health of *recA* mutants suggests that PriA may have a more fundamental role in preventing disruption during normal DNA replication. PriA is required for replication in some reconstituted systems, and its phenotypes in vivo are consistent with the idea that it may contribute to replication processivity or the stability of priming events in some situations.

Although further characterization of the defect responsible for *priA* mutant's poor growth and low viability is required, this does not exclude the likelihood that this protein also participates in the resumption of replication following disruption. Several in vitro experiments have shown that PriA can promote the loading of the replicative helicase, DnaB, at sites such as D-loops (76, 94, 104, 106, 127–129, 198), which could arise at sites of replication arrest or at recombinational intermediates. Based upon these studies, it has been proposed that one possible

mechanism to resume replication when it is blocked by DNA damage would be to reprime the leading-strand template beyond the blocking lesion and re-establish a replication fork from this downstream site. This is a viable model but it remains to be documented in vivo.

CONCLUDING REMARKS

RecA fulfills an essential role in the restoration of processive replication following arrest of the replication fork at sites of DNA damage. That role is likely to vary significantly depending upon the nature of the offending lesion. In the case of UV-induced DNA lesions, RecA functions both to maintain the DNA strands at the fork and to upregulate genes that help remove or tolerate the lesions. The experimental evidence currently supports the idea that excision repair is the primary mechanism to deal with the arresting lesions at blocked replication forks. It also suggests that translesion DNA synthesis can function as a competitive alternative to lesion removal and can additionally function as the primary mechanism for dealing with an as-yet-undefined subset of lesions that are encountered during replication. It will be important in the immediate future to determine the preferred substrates for these respective mechanisms. Furthermore, several experimental observations suggest that recombinational strand exchange is unlikely to play a significant role following UV-induced DNA damage, unless these initial options have failed, and at that point cellular survival will have already been severely compromised.

Although an immense amount of information about the functions of *recA* and other recombination genes has been generated over the past 35 years, the conceptual realization that these genes may function to maintain the strands of genetic information rather than rearrange them during chromosome replication has been relatively recent (26, 84). This novel perspective has generated much discussion and the question of how replication recovers following disruption has received an intensified amount of study over the past six years. With this interest and enthusiasm, there has sometimes been a tendency to implicate (or perhaps overimplicate) many genes in this process, simply because the respective mutants grow poorly or render cells hypersensitive to UV. Although this may represent a good starting point for investigation, the recovery of replication is not the only process required for successful reproduction. Unless growing *E. coli* have been subjected to external agents that inflict replication-arresting damage, it would appear that the functions of RecA are not frequently required, or that some of those functions can be accommodated by other proteins. Several of the other proteins discussed in this review have either been shown to be nonessential for the recovery of arrested replication forks or they have not been tested directly for that process. However, most of these gene products clearly contribute to survival and/or genomic stability when DNA damage is present. In addition, there exist several potential candidate genes that have not yet been considered, including most of the 43 genes that are upregulated in the SOS response following replication arrest, as well as several others that, when mutated, render cells hypersensitive to DNA damage.

The scope of this review has been restricted to the few genes for which there is evidence for a direct involvement in the recovery of DNA replication. Clearly, there are many functions that are essential for maintaining genomic stability and survival in the presence of DNA damage. Other critical phenomena that, to date, have not been extensively investigated, include how nonblocking-DNA lesions are processed at replication forks; how replication accurately completes replication at its doubling point; how the cell senses, partitions, and resolves the chromosomes once replication has been completed; and how the cell maintains access to all regions of the genome. Each of these processes is specific to the DNA metabolism and would not necessarily be expected to affect the recovery of DNA replication following disruption, but would certainly have an impact upon cellular viability in the presence of DNA damage.

Each of these processes invokes highly specialized, although possibly related, enzymatic pathways. We still need to focus upon the special roles that the less-studied genes may play, including those for which we have essentially no information at all. It will be exciting to develop new cellular assays to dissect the many unique problems that are manifested during the cell's attempts to accurately copy and pass on its genomic legacy to the next generation.

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