Therefore, what are recombination proteins there for?

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Summary

The order of discovery can have a profound effect upon the way in which we think about the function of a gene. In *E.coli*, *recA* is nearly essential for cell survival in the presence of DNA damage. However, *recA* was originally identified, as a gene required to obtain recombinant DNA molecules in conjugating bacteria. As a result, it has been frequently assumed that *recA* promotes the survival of bacteria containing DNA damage by recombination in which DNA strand exchanges occur. We now know that several of the processes that interact with or are controlled by *recA*, such as excision repair and translesion synthesis, operate to ensure that DNA replication occurs processively without strand exchanges. Yet the view persists in the literature that *recA* functions primarily to promote recombination during DNA repair. With the benefit of hindsight and more than three decades of additional research, we reexamine some of the classical experiments that established the concept of DNA repair by recombination, and we consider the possibilities that recombination is not an efficient mechanism for rescuing damaged cells, and that RecA may be important for maintaining processive replication in a manner that does not generally promote recombination. *BioEssays* 23: 463–470, 2001. © 2001 John Wiley & Sons, Inc.

The discovery of *recA* and initial observations

The pioneering studies of A. J. Clark and A. D. Margulies,[1] whose goal was to identify of genes required for genetic recombination in *E.coli*, led to the identification of *recA*. The authors defined recombination as,"the inheritance by recombinant progeny of double-stranded elements of DNA derived from two parents" and they employed an Hfr conjugation assay in which recombinants could be selected based upon the inheritance of growth properties from each parent. By identifying mutants that failed to form recombinants during mating, they discovered *recA*. They went on to show that, although DNA was transferred from donor to recipient cells during conjugation, no recombinants were obtained from *recA* mutants, thereby demonstrating that some function of *recA* was required for the formation of recombinant DNA molecules.

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The concept of recombination as a repair mechanism

Clark and Margulies,[1] following up on ideas developed by Paul Howard-Flanders, also made the important observation that their recombination-deficient mutants were hypersensitive to UV light. Because of the sensitivity of *recA* mutants to UV and ionizing radiation, Howard-Flanders proposed that DNA repair and recombination involve common enzyme-mediated steps.[2,3] Furthermore, because a *recA* mutation decreased the resistance of *uvrA* mutants to UV irradiation[3,4] (Fig. 1A), he concluded that the survival promoted by the wild-type *recA* allele was distinct from excision repair and could be due to a recombinational mechanism of repair (Fig. 2A). Based upon this interpretation, a large number of subsequent studies began with the assumption that wild-type *recA* promotes recombinational repair, and then went on to characterize the molecular events occurring in *uvr* mutants with the belief that they must represent this recombination-dependent repair pathway (reviewed in Ref. 5). These studies revealed that the replication occurring in *uvr* mutants after UV irradiation was accompanied by high frequencies of DNA strand exchanges (Fig. 3A). Furthermore, the DNA synthesized after UV irradiation was initially in the form of short fragments that were subsequently converted to larger molecules resembling normal DNA in size.[6–8] Since these events were assumed to represent repair, a model was proposed in which the RecA gene product promoted recombination as a mechanism to reconstruct functional genomes from the discontinuous daughter strands and undamaged regions of the parental strands[6,7] (Fig. 2A). Due to the hypersensitivity of *recA* mutants to UV, the general view evolved that the proposed recombinational mechanism must represent a major repair pathway, required for cellular survival and genomic stability.[9]

This intriguing proposal established what came to be the primary assumption in many subsequent studies. Unquestionably this line of investigation has provided an immense amount of information about the genetic elements involved in

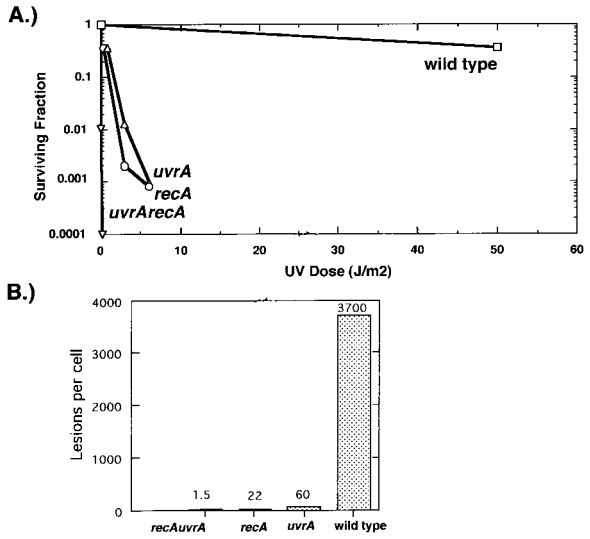

Figure 1. A: The survival of various *E. coli* mutants following UV irradiation (adapted with permission from Refs 3 and 4). **B:** The numbers of cyclobutane pyrimdine dimers required to produce an average of one lethal lesion per bacterium were taken from Ref. 3 and are shown in the figure.

recombination. Today, however, we know much more about RecA function. For example, we now know that RecA itself is central to the regulation of more than thirty genes constituting the SOS response (reviewed in Ref. 10). When the functions of the genes that are regulated by RecA are examined, one is struck by the observation that most of them have nothing to do with recombination. In fact, many seem to be involved with the task of restoring processive DNA replication. Among the genes included in the SOS response are *uvrA* and *uvrB*, the products of which are required for removing UV-induced lesions from DNA by nucleotide excision repair. Recently, upregulation of these genes was shown to be critical for efficient repair of cyclobutane pyrimidine dimers, the major photoproduct induced by UV irradiation.[11] Clearly, the idea that excision repair is independent of *recA* is no longer tenable. Other genes upregulated by RecA include *polB, dinB*, and *umuC*. These genes encode DNA polymerases (Pol II, Pol IV, and Pol V, respectively) that facilitate processive replication, or bypass, through lesions that would otherwise block replication.[12,13] Lesion bypass itself would be expected to reduce the need for recombination events but, in addition, the UmuD'C complex interacts with RecA which may target it to lesions and facilitate bypass.[14,15]

In this context, observations from human cells belonging to the xeroderma pigmentosum variant (XPV) complementation group may be instructive. These cells are moderately sensitive to UV, but are not deficient in nucleotide excision repair. Following UV irradiation, DNA synthesis is inhibited and abnormally short DNA fragments are produced which mature into larger molecules very slowly. These observations initially led to the interpretation that XPV cells were defective in recombinational repair. However, more recent studies and the cloning of the XPV gene has shown that the gene product is a DNA polymerase, pol ε, which belongs to the same family as the UmuC and DinB proteins and allows replication to continue processively through DNA lesions that would otherwise block replication.[16–18]

Considering these observations, we believe that it is appropriate to re-examine the question, "Is recombination an efficient mechanism for repairing DNA damage?" If the authors of the original studies had the benefit of current knowledge about *recA* function and DNA replication, they might have suggested that recombination is not a predominant repair pathway, a reasonable conclusion considering the dramatic UV sensitivity of nucleotide excision repair-deficient cells compared to wild type (Fig. 1A). Previous interpretations focused upon the survival promoted by the wild-type allele of *recA* in excision repair-deficient mutants of *E. coli*. However, the *uvr* (Rec+) mutants only survive UV doses producing approximately 60 lesions per genome, while the wild-type parental strain recovers from UV doses producing in excess of 3500 lesions per genome (Fig. 1B). From a practical point of view, these results demonstrate that, in the absence of nucleotide excision repair, *recA* function does not contribute significantly to cellular viability. Because even very low levels of DNA damage generate high levels of lethality and mutagenesis in excision repair-deficient populations, one might conclude that the DNA strand exchanges observed in *uvr* mutants are just as likely to be scrambling the genome as they are to be productively reconstructing it.

Adding excision repair to the equation

Perhaps the poor survival of *uvr* mutants in which *recA* is fully functional suggests that RecA does not normally promote recombination to circumvent DNA lesions but rather is needed to maintain replication forks that are blocked by DNA lesions until those lesions can be removed by excision repair as shown in Fig. 2B. If the offending lesion cannot be removed by excision repair, then survival is poor inspite of any recombination that occurs.

This manner of thinking may also better explain the inhibition of replication that occurs after UV irradiation in *recA* mutants.[4,19,20] In both *recA* and wild-type cells, DNA lesions induced by low doses of UV are removed by nucleotide excision repair.[21] If the classic recombinational repair model shown in Fig. 2A were efficient, one would expect the rates of DNA replication in UV-irradiated wild-type and *recA* cells to be similar; however, the impaired replication in *recA* mutants is not consistent with this idea.

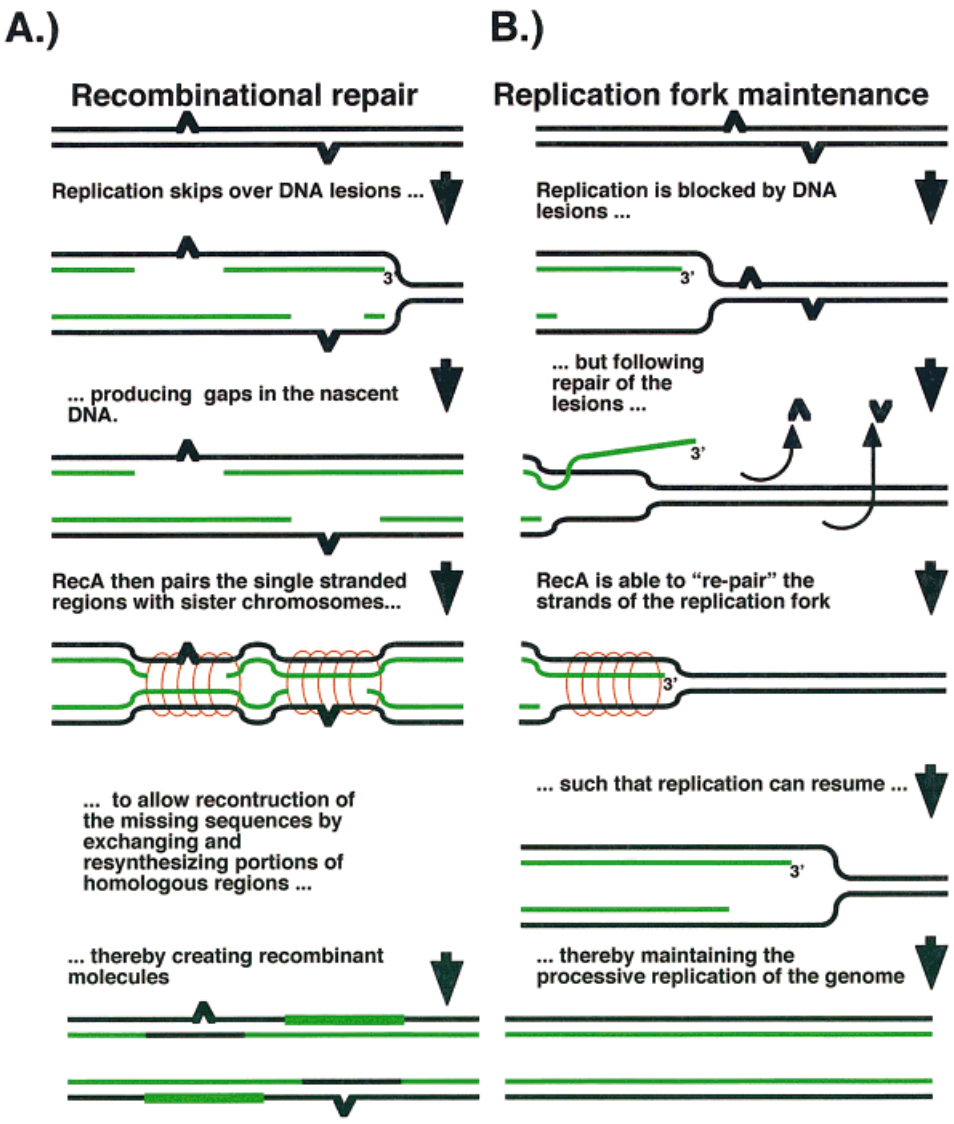

Figure 2. Comparing **A:** the classical recombinational repair model (daughter strand gap repair) to **B:** one in which RecA is proposed to maintain the replication fork when it is blocked by DNA damage.

It seems to us that a most important observation with respect to RecA function during replication comes from the early studies of Horii and Suzuki.[22] They observed that a rapid degradation of the genome occurred in the *recA* mutants, something that did not occur in either wild-type or *uvrA* mutants. Furthermore, they found that this degradation was more pronounced if the *recA* mutants were actively replicating DNA at the time of exposure than if they were not (Fig 4). Based on results of their pulse-chase labeling experiments, they concluded that the degradation initiated from the replication forks and proceeded back from these points. Their observations led them to propose that RecA function was required to protect the DNA at replication forks blocked by DNA damage, a concept very similar to that represented in Fig. 2B.

A function of RecA common to recombination and replication

The proposal of Horii and Suzuki[22] that RecA can protect replication forks is important and provocative because it could explain why RecA is required when the DNA is damaged. Significantly, their proposal also suggests that RecA is acting

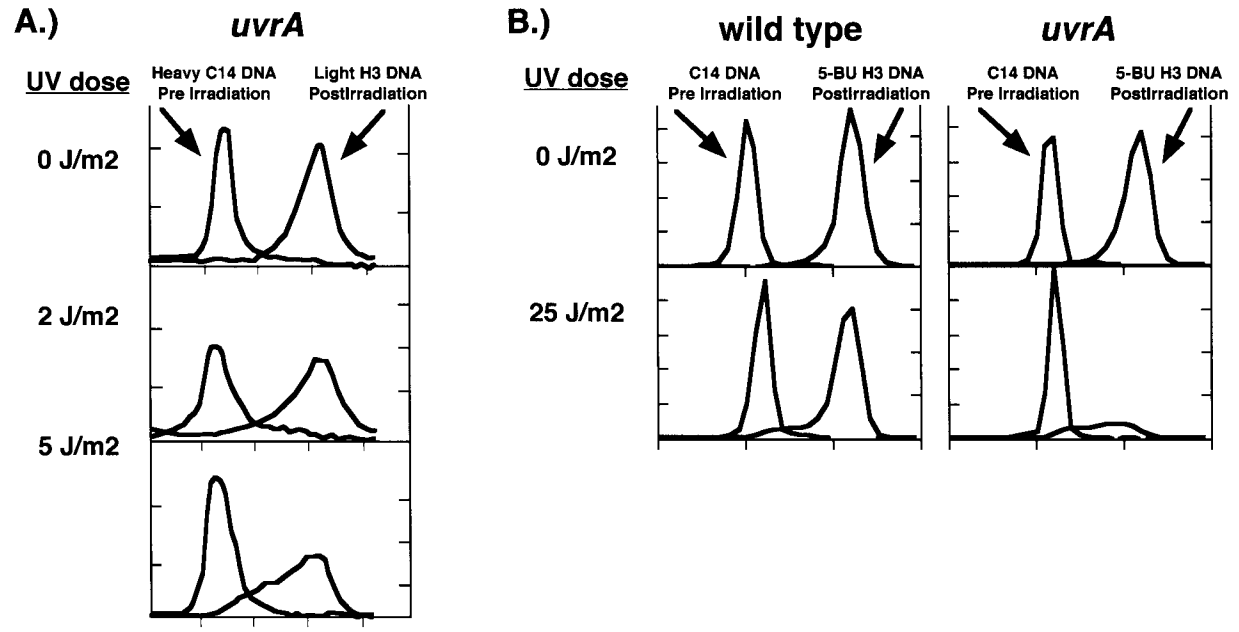

Figure 3. A: Experiment classically interpreted to suggest that *recA* promotes recombinational repair during replication (adapted with permission from Ref. 7). By growing cells in different isotopic media before and after irradiation, the DNA made before and after irradiation can be separated by differences in their buoyant density in isopycnic CsCl gradients. To test whether recombination can be induced by UV lesions, *uvr* mutants were irradiated with low UV doses and allowed to recover for an hour. Compared to unirradiated *uvr* mutants, the DNA made by the irradiated cultures contained more DNA of an intermediate density, an observation that has been interpreted to represent exchanges between parental and daughter DNA and as evidence that recombinational repair is occurring. **B:** If, however, one compares the profiles obtained from wild-type cells to those from *uvr* mutants in this type of experiment, one finds that replication is severely inhibited in the *uvr* mutants and that replication does not recover normally. In the original study, which used only *uvr* mutants, although exchanges could be detected following a dose of 5 J/m^2, no further growth was observed in this population of cells.

upon a common substrate during both recombination and replication. In vitro, purified RecA progressively binds and pairs single-strand DNA with homologous duplex DNA in a 5′ to 3′ direction. The product of this reaction creates a RecA protein filament bound to a triple-stranded DNA structure (for reviews see Refs. 23–25). During recombination, this activity is thought to be critical for bringing together homologous strands from different DNA molecules (Fig. 5A). During DNA replication, however, this *same* biochemical activity of RecA may be required for a nonrecombinational role at the replication fork (Fig. 5B). Semiconservative replication copies both strands of the DNA template concurrently in a 5′ to 3′ direction. While the leading strand can be synthesized continuously, synthesis on the lagging strand template occurs discontinuously, periodically reinitiating as the replication machinery moves processively along the template. The coordination of this process implies that, at any given time, the region immediately behind the replication machinery will contain a single-stranded region. If replication becomes blocked, the region behind the replication fork will be essentially identical to the substrate upon which RecA has been shown to function during recombination (Fig. 5B). In this case, however, pairing between the duplex strands on the leading strand side of the fork with the single-strand template on the lagging strand side of the fork might be expected to maintain, rather than rearrange, the strands of the replication fork. As mentioned earlier, the lack of viability in the absence of excision repair may indicate that such pairing stabilizes the fork until the offending lesion has been repaired, thereby preserving the accurate and processive duplication of the template (Fig. 2B) but contributing little to survival if the lesion cannot be removed.

Events occurring at blocked replication forks

Similar to the case of *recA*, the influence that a gene's name can have upon functional considerations may be found among the genes in the *recF* pathway in *E. coli*. These genes (*recF*, *recO*, *recR*, *recJ*, *recQ*) were classified as recombination genes based upon the criterion that they are specifically required for congujational recombination when the *recBCD*

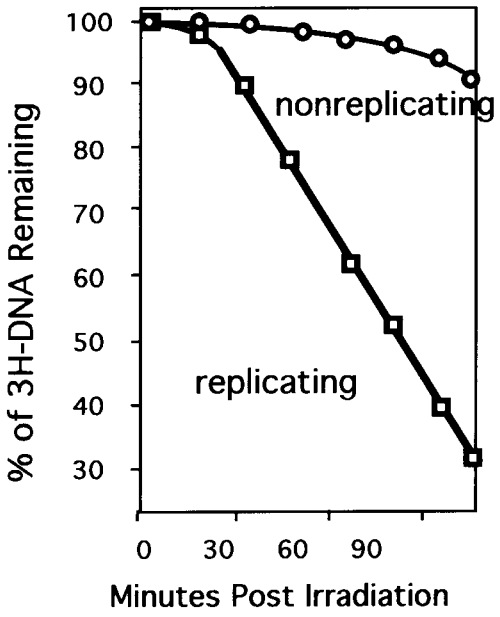

Figure 4. Genomic degradation in *recA* mutants following 5 J/m^2 UV irradiation. The degradation of DNA made prior to UV irradiation in a *recA* mutant was determined by the amount of acid-precipitable radioactivity remaining. Replicating or non-replicating *recA* mutants were grown in the presence of [^{3}H]thymidine, then placed into nonradioactive medium and UV irradiated with 5 J/m^2. (Adapted with permission from Ref. 22).

recombination pathway is inactivated.[26] As a result, studies have always assumed that RecF promotes recombination (reviewed in Refs. 5,23,24,27,28). In fact, initial studies found that purified RecF *inhibited* RecA-mediated strand exchange, but this was interpreted to be functionally unrepresentative and subsequent studies focused upon finding conditions in which inhibition did not occur.[29–31]

Alternatively however, one might consider that, in an otherwise wild-type background, *recF* pathway mutants undergo conjugational recombination normally.[26,32] Yet, despite this recombinational proficiency, *recF, recO, recR* mutants are hypersensitive to UV irradiation.[26,32] Similarly, certain *recA* mutants retain the capacity for some kinds of recombination, but show hypersensitivity to UV even when the SOS functions are induced.[33,34] These observations suggest to us that the UV sensitivity of these mutants may result from defects in some process other than recombination.

With this perspective in mind, it is interesting to note that the recovery of replication in UV-irradiated *recF* mutants is severely inhibited and localized degradation occurs at the replication fork.[35] Furthermore, as with *recA*, the recovery promoted by *recF* depends upon *uvrA*, and may therefore require removal of the blocking lesions.[20] These observations suggest that RecF, like RecA, is required at blocked replication forks for the resumption of replication to occur.[20,35,36]

In focusing upon the molecular events occurring at blocked replication forks, our recent studies revealed that, when replication is blocked by DNA damage, limited degradation of the nascent DNA can also be detected in wild-type cells.[20,36] Characterization of this nascent DNA degradation demonstrated that other *recF* pathway genes, *recJ* and *recQ,* are responsible for the degradation and appear to degrade the nascent lagging strand preferentially, as shown in Fig. 6.[36] These results are particularly interesting because, although these genes have been primarily characterized for recombinational phenotypes, RecQ homologs in yeast, *Drosophila*, and humans have been found to play critical roles in maintaining processive replication and suppressing strand exchanges from occurring (reviewed in Ref. 37). In *E. coli*, RecQ increases the single-strand region at blocked replication forks, creating a much larger substrate upon which the RecA protein may bind and stabilize, perhaps preventing recombination from occurring in these situations.

Conclusions

Halting and resuming processive replication has a symmetry that we feel has more potential, from enzymatic considerations, of generating precisely two complete genomes than do attempts to unscramble DNA strands knitted together by the exchanges envisioned in models of recombinational repair.

With this view in mind, it will be interesting and necessary to scrutinize the role that other genes, classically implicated in recombination, have in the replication process. For instance, an interesting question arises as to how nucleotide excision repair gains access to a lesion that may be hidden within the blocked replication machinery. One might expect that, for the repair machinery to operate on the DNA lesion, the replication machinery and nascent DNA will first have to be transiently displaced. In the case of transcription, there is good experimental evidence that an RNA polymerase blocked at a DNA lesion prevents repair enzymes from getting to the blocking lesion.[38–41] To effect repair in *E. coli*, a special helicase-like protein, encoded by *mfd*, can displace the RNA polymerase and transcript and facilitate repair.[40,42] In *mfd* mutants, transcription-blocking lesions are not rapidly repaired and the cells are moderately UV sensitive. Similarly, when DNA replication is blocked by a lesion, a helicase such as RecG might help to displace the replication machinery and nascent DNA to effect repair. It is worth noting that the RecG catalyzes branch migration upon three-stranded substrates with polarities that would be required for this function.[43] Also of interest is the observation that *recG* shares a significant degree of homology with *mfd*. In addition, mutations that inactivate *recG* confer a moderate degree of hypersensitivity to UV irradiation.[44] For this reason, we have included RecG in our model in

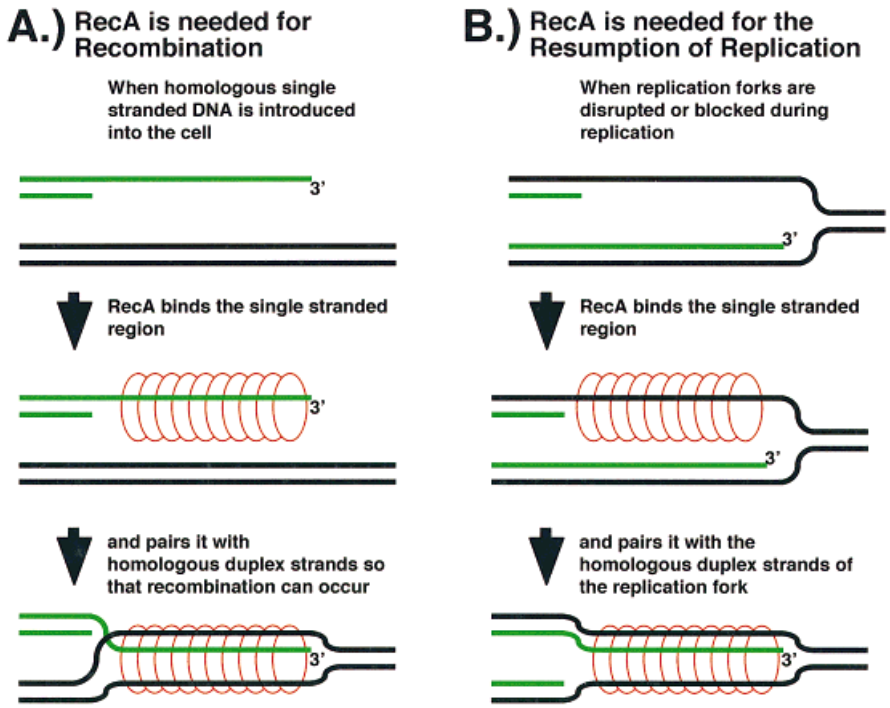

Figure 5. Comparing the structures used to initiate recombination to that of the replication fork. **A:** Genetic recombination in *E. coli* appears to require a 3′ single-strand segment of DNA. When this form of DNA is introduced or generated inside the cell, RecA is thought to initiate the recombinational process by binding this single-strand region and pairing it with homologous duplex DNA. The paired DNA can then be fixed into the genome by replication or resolution of the crossover point (Holliday junctions) to generate a recombinant molecule. **B:** During semiconservative replication, concurrent DNA synthesis in a 5′ to 3′ direction on both strands of the DNA template creates a single-stranded region on the lagging strand template. Thus if replication is prematurely disrupted or blocked, the nascent DNA is expected to terminate with a 3′ end on the leading strand and a 5′ end on the lagging strand, as shown. Unlike recombinational processes, however, binding of RecA to the single-stranded region at the replication fork and pairing it with the homologous duplex DNA will help maintain the processive semiconservative replication of the genome and would not create a recombinant molecule.

Fig. 6; however, no direct experimental test of RecG in replication recovery has yet been reported.

Although we have been discussing types of exogenous DNA damage (i.e. UV photoproducts) that do not directly cause strand breaks, some estimate of the frequency of replication disruption in unirradiated cells can be inferred from the initial studies of Howard-Flanders and coworkers who found that, in the absence of nucleotide excision repair, a UV dose producing a single lesion is sufficient to kill a *recA* mutant.[2,4] If RecA is absolutely required for replication resumption at even a single lesion, the relative health and viability of *recA uvrA* mutants in the absence of exogenous damage would suggest that, more often than not, these events do not occur. This observation is important because many current models use the poor viability of "recombination-deficient" mutants as evidence that recombination must be an essential and frequent event occurring during replication.[5,25,45–47] However, since *recA* is absolutely required for recombination, the fact that the viability of *recA* mutants is better than that of many of the other "recombination-deficient" mutants, such as *recBC* or *priA*,[48–50] suggests that the viability problems of these other mutants must extend beyond the phenomenon of recombination.

Thus it may be time to reevaluate the contribution that recombination makes to the viability of injured *Escherichia coli* and to reassess the role of recombination proteins in DNA replication. To us, genomic replication appears to accomplish its monumental task of duplicating multimegabase chromosomes by maintaining a symmetry throughout this process. While "recombination" proteins are indeed central to this process, it is worth reexamining their behavior, avoiding preconceptions, to discern precisely how they function.

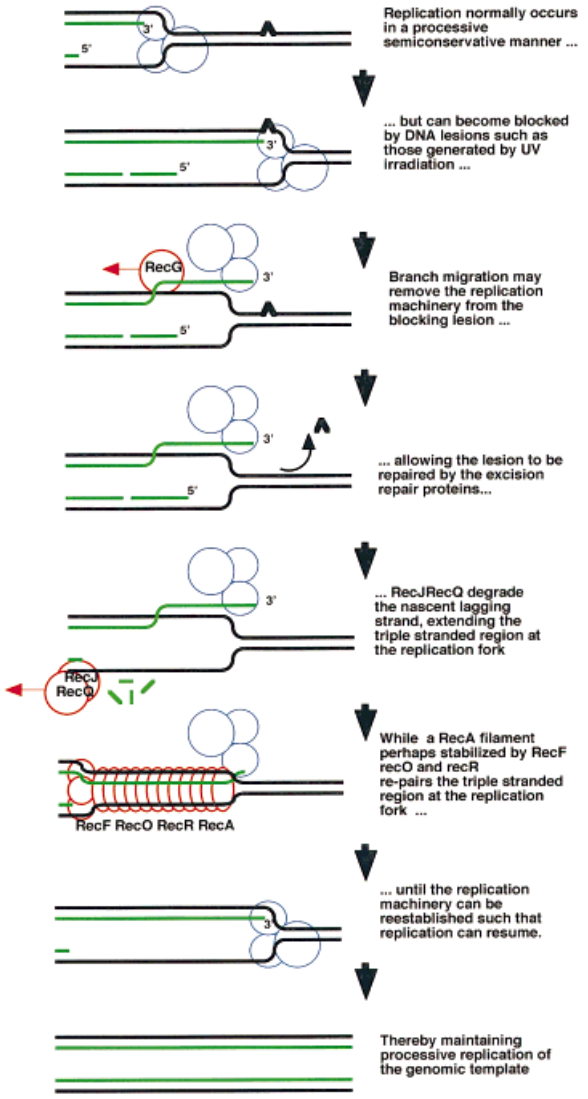

Figure 6. Detailed proposal for the recovery of replication that encounters a blocking DNA lesion.

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