Mol Gen Genet (1999) 262: 543–551

ORIGINAL PAPER

J. Courcelle · P. C. Hanawalt

RecQ and RecJ process blocked replication forks prior to the resumption of replication in UV-irradiated *Escherichia coli*

Received: 1 June 1999 / Accepted: 28 July 1999

Abstract The accurate recovery of replication following DNA damage and repair is critical for the maintenance of genomic integrity. In *Escherichia coli*, the recovery of replication following UV-induced DNA damage is dependent upon several proteins in the *recF* pathway, including RecF, RecO, and RecR. Two other *recF* pathway proteins, the RecQ helicase and the RecJ exonuclease, have been shown to affect the sites and frequencies at which illegitimate rearrangements occur following UV-induced DNA damage, suggesting that they also may function during the recovery of replication. We show here that RecQ and RecJ process the nascent DNA at blocked replication forks prior to the resumption of DNA synthesis. The processing involves selective degradation of the nascent lagging DNA strand and it requires both RecQ and RecJ. We suggest that this processing may serve to lengthen the substrate that can be recognized and stabilized by the RecA protein at the replication fork, thereby helping to ensure the accurate recovery of replication after the obstructing lesion has been repaired.

Key words Replication · DNA repair · *recJ* · *recQ* · UV irradiation

Introduction

All cells must faithfully replicate their genomes in order to reproduce. However, if not repaired, DNA damage that blocks replication can lead to a loss of genomic stability, mutations, or cell death. Despite the importance of the process by which replication recovers, the cellular mechanism(s) by which this occurs in DNA repair proficient cells remains largely uncharacterized.

Irradiation of cells with near UV light induces lesions in the DNA which block replication. In *E. coli*, replication is transiently inhibited following a moderate dose of UV irradiation, but it efficiently recovers following the removal of the UV-induced lesions (Setlow et al. 1963; Setlow and Carrier 1964; Courcelle et al. 1998). The efficient recovery of replication in wild-type cells is accompanied by the partial degradation of the nascent DNA at the replication fork prior to the resumption of DNA synthesis (Hanawalt and Brempelis 1967; Courcelle et al. 1997, 1998). However, it is not known whether this degradation is required for, or contributes in any way to, the normal recovery process.

The resumption of replication following UV-induced DNA damage is largely dependent upon the removal of the lesions by nucleotide excision repair (Setlow et al. 1963; Courcelle et al. 1998). However, a large body of work with repair-deficient mutants has shown that UV irradiation can lead to recombination events when replication forks encounter DNA damage that cannot be repaired (Rupp and Howard-Flanders 1968; Rupp et al. 1971; Ganesan and Smith 1971; Ganesan 1974). In these mutants, the recovery of replication is severely inhibited, resulting in loss of semiconservative replication, high frequencies of chromosomal exchanges, and extensive cell death (Setlow et al. 1963; Howard-Flanders et al. 1969; Rupp et al. 1971; Ganesan 1974; Courcelle et al. 1997, 1998). In contrast, these recombination events are efficiently suppressed in normal, repair-proficient cells; survival is greatly enhanced and the recovery of replication is much more efficient, suggesting that the normal mechanism of recovery may be quite different from that observed in repair-deficient mutants (Courcelle et al. 1997, 1998).

In addition to removal of the lesions, however, the recovery of replication also requires the function of RecA and the *recF* pathway proteins (Templin et al. 1972; Horii and Clark 1973). Historically, because most of these proteins were identified through recombination

Communicated by R. Devoret

J. Courcelle (✉) · P. C. Hanawalt
Biological Sciences, Stanford University,
Stanford, CA 94305, USA
E-mail: jcc@leland.stanford.edu;
Fax: +1-650-7251848

assays, their functions have been assumed to be specific to recombination. However, in the presence of DNA damage, these genes are also required for genomic replication to occur (Clark and Margulies 1965; Howard-Flanders et al. 1969; Horii and Clark 1973; Courcelle et al. 1997, 1998). Whereas these genes were identified because of their roles in the creation of recombinant progeny, genomic replication in bacteria normally creates clones of cells which have identical genomes. The daughter genomes are generated through a processive, semiconservative replication of the parental genomic template (replicated regions which have undergone strand exchanges would not be semiconservative). The general lack of recombination which is observed during replication in asexual reproductive cycles suggests that, although these *rec* genes are necessary for recombinational processes to occur, they may be required for a different purpose during genomic replication.

One alternative function is suggested by several studies which demonstrate that these gene products are needed to stabilize and maintain the strands of the advancing replication forks when they are blocked by DNA damage, rather than to promote DNA strand exchanges as occurs during recombination. The association with replication forks was initially inferred from the observation that, although UV-induced lesions are removed from the DNA in *rec* mutants at rates which are similar to those in wild-type cells, replication does not resume (Clark and Chamberlin 1966; Rothman 1978; Courcelle et al. 1998). In addition, in *recA* mutants, it has been shown that when replication encounters a UV lesion, a rapid and eventually complete degradation of the entire genome occurs (Horii and Suzuki 1968; our unpublished observations). However, in either the presence of RecA or in the absence of replication, the genomic DNA remains protected (Horii and Suzuki 1968; our unpublished observations). Furthermore, the degradation which occurs in the absence of RecA has been shown to initiate at the blocked replication forks and processively regress back from these points (Horii and Suzuki 1968). These observations are all consistent with the idea that RecA function is required to stabilize and protect the strands of the replication forks which are blocked by DNA damage.

Cells with mutations that inactivate *recF*, *recO*, or *recR* also fail to recover replication following UV irradiation (Horii and Clark 1973; Kolodner et al. 1985; Mahdi and Lloyd 1989; Courcelle et al. 1997). Following irradiation of these mutants, the genomic DNA remains largely intact. However, extensive degradation of the nascent DNA occurs, suggesting that the *recF* deficiency relates more specifically to an inability to recognize, or resume replication from, the blocked replication forks. In UV-irradiated wild-type cells, the degradation of nascent DNA is limited and occurs prior to the time at which replication recovers, suggesting that this degradation may be a normal part of the recovery process (Courcelle et al. 1997, 1998). These observations have led to our proposal that a primary role of RecA and the *recF* pathway proteins during the asexual replication of the bacterial genome is to maintain blocked replication forks until replication can resume after the DNA damage has been repaired (Courcelle et al. 1997, 1998). Purified RecA protein has been shown to bind single-stranded DNA and to pair it with homologous duplex DNA, an activity which is thought to be critical in bringing together homologous pieces of DNA during recombination (for reviews see Smith 1988; Kowalczykowski et al. 1994; Roca and Cox 1997). However, during genomic replication, this same enzymatic activity could also function to protect and maintain the pairing between the strands of the replication fork until the block to replication has been removed.

Both RecQ (a 3′→5′ DNA helicase) and RecJ (a 5′→3′ single strand-specific exonuclease) belong to the *recF* pathway, although, unlike RecF, the absence of these proteins does not prevent the recovery of replication (Lovett and Clark 1984; Nakayama et al. 1984; Lovett and Kolodner 1989). However, mutations which inactivate RecJ have been shown to alter the sites and frequency of illegitimate rearrangements following DNA damage (Ukita and Ikeda 1996). *recQ* mutants show an *increase* in the frequency of illegitimate rearrangements, suggesting a possible role for RecQ in preventing rearrangements during the recovery process (Hanada et al. 1997).

In other organisms, RecQ homologs have been shown to have a similar role in preventing strand exchanges and maintaining semiconservative replication. In humans, mutants in BLM fail to maintain semiconservative replication, displaying high rates of sister chromatid exchanges (Langlois et al. 1989; German 1995). In *S. pombe*, Rqh-1 is required to suppress recombination, which otherwise leads to an irreversible S-phase arrest (Stewart et al. 1997). In *S. cerevisiae*, *sgs1* mutants display higher frequencies of chromosome nondisjunction following mitosis (Stewart et al. 1997).

In trying to understand the mechanism by which accurate replication is maintained in the presence of DNA damage, we have further characterized the degradation of nascent DNA which occurs prior to the resumption of replication in UV-irradiated *E. coli*. We find that the degradation occurs on the nascent lagging strand of the replication fork and requires functional copies of *recQ* and *recJ*. We present a model to explain how this processing could function to help RecA filaments stabilize and maintain the strands at the blocked replication fork until the obstructing lesion has been repaired.

Materials and methods

Bacterial strains

SR108 is a *thyA36 deoC2* derivative of W3110 (Mellon and Hanawalt 1989). HL946 (SR108 *recF332*::Tn*3*), HL924 (SR108

*recJ*284::Tn*10*) and HL944 (SR108 *recQ1803*::Tn*3*) were made by P1 transduction of the *recF332*::Tn*3*, *recJ284*::Tn*10*, and *recQ1803*::Tn*3* markers from strains HL556, JC12123, and KD2250, respectively, into SR108. HL1034 (SR108 *xonA*::Cat*300*), HL922 (SR108 *recB21 recC22 argA81*::Tn*10*), and HL923 (SR108 *recD1011 argA81*::Tn*10*) were made by P1 transduction of the *xonA*::Cat*300*, *recB21 recC22 argA81*::Tn*10*, and *recD1011 argA81*::Tn*10* markers from strains HR838, V1307, and V220, respectively, into SR108. HL973 (SR108 *recF332*::Tn*3*; *recJ284*::Tn*10*), HL1036 (SR108 *recF332*::Tn*3*; *recD1011 argA81*::Tn*10*), and HL1035 (SR108 *recF332*::Tn*3*; *xonA*::Cat*300*) were made by P1 transduction of *recJ284*::Tn*10*, *xonA*::Cat*300*, and *recD1011 argA81*::Tn*10*, respectively, into HL 946. The *recJ* and *recQ* phenotypes were confirmed by their resistance to thymineless death (Nakayama et al. 1985). Three independent isolates of *xonA* mutants were used in these experiments to minimize the chance of illegitimate P1 transductions. Confirmation of the phenotypes of all other mutants used in this study have been reported previously (Courcelle et al. 1997, 1998). KD2250 was a gift from H. Nakayama. JC12123 was a gift of A. J. Clark. HR838 was a gift from S. M. Rosenberg.

DNA degradation following UV irradiation

A 100-µl aliquot of a fresh overnight culture was used to inoculate 10 ml of Davis medium supplemented with 0.4% glucose, 0.2% casamino acids, and 10 µg/ml thymine (DGCthy medium) containing 0.2 µCi of [^{14}C]thymine/ml. Cells were grown in a shaking water bath at 37° C to an OD_{600} of between 0.3 and 0.4 (approximately 5×10^8 cells/ml). At this time, 1 µCi of [^{3}H]thymidine/ml was added to the culture. After 10 s, the cells were filtered through a Whatman HA filter (4.5 cm diameter, 0.45 µm pore size) . The cells were then washed with 5 ml and then 2 ml of NET buffer (100 mM NaCl, 10 mM TRIS-HCl pH8.0, 10 mM EDTA). Washing times varied between 10 and 30 s depending on the cell line. The cells were then resuspended in nonradioactive DGCthy medium and irradiated with a dose of 25 J/m^2 unless otherwise indicated. The elapsed time between transfer of the cells into nonradioactive medium and irradiation was approximately 10 s. The ^{14}C and ^{3}H remaining in the DNA was measured by averaging duplicate, 0.2-ml samples precipitated in 5% cold trichloroacetic acid and filtered onto Whatman glass fiber filters. All zero time points were taken in triplicate rather than duplicate. The initial values for ^{14}C and ^{3}H were between 900–2000 cpm and 5000–12000 cpm in all experiments.

Degradation of nascent DNA in the leading and lagging strands of the *lacZ* gene

An 0.3-ml aliquot of a fresh overnight culture was used to inoculate 30 ml of DGCthy medium containing 0.1 µCi/ml [^{14}C]thymine. The culture was grown to an OD_{600} of between 0.3 and 0.4 before harvesting by filtration and resuspending in DGC medium containing 20 µg/ml 5-bromouracil supplemented with 1 µCi/ml [^{3}H]5-bromodeoxyuridine (Moravek Biochemical). Within 30 s, the culture was filtered again. The cells were then washed with 10 ml of NET buffer, resuspended in nonradioactive DGCthy medium, and irradiated with 25 J/m^2. At the indicated time, 10 ml of cells was placed in an equal volume of ice-cold NET buffer, pelleted and lysed in 0.4 ml 0.5 M K_3PO_4 (pH 11.5). The solution was then subjected to isopycnic alkaline CsCl gradient sedimentation as described previously (Smith et al. 1981). Fractions (30) were collected onto Whatman No.17 paper, and the ^{14}C and ^{3}H in each fraction was determined by scintillation counting. The peak ^{3}H fractions for each time point were applied in triplicate, twice, onto Hybond N+ membrane, using a slot blot apparatus. The samples were then probed for either the nascent leading strand DNA (transcribed strand probe) or nascent lagging strand DNA (non-transcribed strand probe) of pZH-10 as described previously (Mellon and Hanawalt 1989).

Results

The degradation of nascent DNA requires *recJ* and *recQ*

Nearly 100% of the cells in an exponentially growing culture of *E. coli* survive a 25 J/m^2 dose of short-wavelength UV radiation. Although ongoing DNA replication is transiently disrupted at this dose, it efficiently resumes following the repair of the UV-induced lesions (Courcelle et al. 1998). However, prior to the resumption of DNA synthesis, some degradation of the nascent DNA occurs, as we have previously reported (Courcelle et al. 1997, 1998). To examine this degradation, cultures grown in [^{14}C]thymine were pulse labeled with [^{3}H]thymidine for 10 s, transferred to nonradioactive medium, and irradiated with 25 J/m^2 UV. The amounts of ^{3}H and ^{14}C remaining in the DNA were then followed over time. The ^{14}C label allowed us to compare the degradation occurring in the genome overall to that involving the newly synthesized ^{3}H-labeled DNA at replication forks. A typical experiment is shown in Fig. 1. No degradation of the bulk genomic DNA is detected, regardless of whether the cells were irradiated or not. No degradation of the nascent DNA is detected in unirradiated cultures after the remaining intracellular pools of [^{3}H]thymidine are rapidly incorporated into the DNA. However, in UV-irradiated cultures, the nascent DNA is partially degraded prior to the recovery of replication. The increase in ^{3}H-labeled DNA after 60 min is probably due to the incorporation of the remaining intracellular pools of [^{3}H]thymidine when replication resumes.

When the nascent DNA from irradiated *recJ* or *recQ* mutants was examined, no degradation was observed.

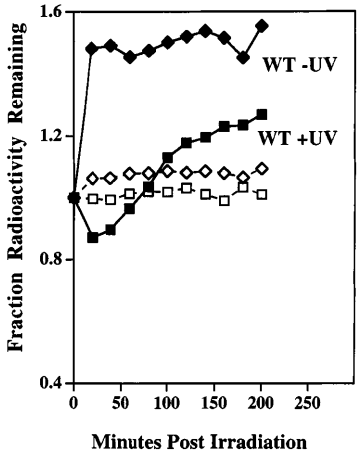

Fig. 1 Degradation of nascent DNA following UV irradiation. [^{3}H]Thymidine was added to cells prelabeled with [^{14}C]thymine 10 s before the cells were placed in nonradiolabeled medium and either UV irradiated with 25 J/m^2 or left unirradiated. The relative amount of radioactivity remaining in the DNA is plotted over time. *Open symbols*, ^{14}C-labeled (genomic) DNA; *filled symbols*, ^{3}H-labeled nascent DNA at the growing fork (labeled on graph); *squares*, irradiated cells; *diamonds*, unirradiated cells

Figure 2A shows the degradation pattern of mutant strains which were processed in parallel with the wild-type cells shown in Fig. 1. The lack of [^{3}H]thymidine incorporation seen in the period immediately following irradiation in *recJ* or *recQ* mutants (Fig. 2A) suggests that the effect is due to a lack of degradation rather than a failure of replication to arrest at sites of DNA damage. That replication is still inhibited by DNA damage in *recJ* and *recQ* was confirmed in experiments in which irradiated and unirradiated *recJ* and *recQ* mutants were compared (data not shown). In contrast to the effect seen with *recJ* and *recQ*, mutants in *xonA* or *recD*, which encode other DNA exonucleases, or *recBC*, which encodes another DNA helicase, did not prevent the degradation of nascent DNA from occurring (Fig. 2B). Other mutations examined that did not abrogate the degradation of the nascent DNA included *recG*, *mfd*, *uvrA*, and *uvrC* (Courcelle et al. 1998 and data not shown). The amount of degradation in individual experiments typically ranged from 10 to 20% of the nascent DNA in these strains. Whenever less than 20% degradation of nascent strands was observed, a correspondingly higher level of reincorporation of [3H] was observed once replication recovered, suggesting that the remaining intracellular pool from the [^{3}H]thymidine pulse was the limiting factor in detecting the degradation. Furthermore, the observed reincorporation following the resumption of replication suggests that the actual amount of degradation detected may be an underestimate of that actually occurring at the replication fork. However, comparisons between mutants from individual experiments always showed the same differences relative to each other in at least two independent experiments.

Degradation occurs on the nascent lagging strand

Unlike wild-type cells, *recF* mutants fail to recover replication following interruption by UV irradiation, despite the fact that the removal of UV-induced lesions occurs with kinetics similar to those in wild-type cells (Rothman 1978; Courcelle et al. 1998). In the *recF* mutants, the degradation of nascent DNA continues beyond the time at which replication recovers in wild-type cells, but ceases after 50% of a 10-s pulse label has been degraded (Fig. 3A) (Courcelle et al. 1997, 1998). The slight loss of the label in bulk genomic DNA seen in the *recF* mutants occurs primarily at later times and is thought to be due to secondary effects following the failure of replication to recover normally.

When we examined a *recF recJ* double mutant, no degradation of the nascent DNA was detected. This suggests that the degradation of nascent DNA that occurs in *recF* mutants, although more extensive, is likely to be mechanistically similar to that which normally occurs in wild-type cells (Fig. 3A).

As was found with wild-type cells, mutations in either *recD* or *xonA* also did not alter the pattern of degradation in *recF* mutants (Fig. 3B). The degradation of nascent DNA in *recR* mutants, which is similar to that in *recF* mutants, was also found to be dependent upon *recJ* and *recQ*, but was unaffected by mutations in *recD*, *recG*, or *mfd* (data not shown).

We were interested in the observation that although replication does not recover in *recF* mutants, the nascent DNA degradation appears to be limited to approximately 50% of the nascent DNA. Considering the complementing polarities of RecJ and RecQ, we reasoned that the degradation might be limited to one

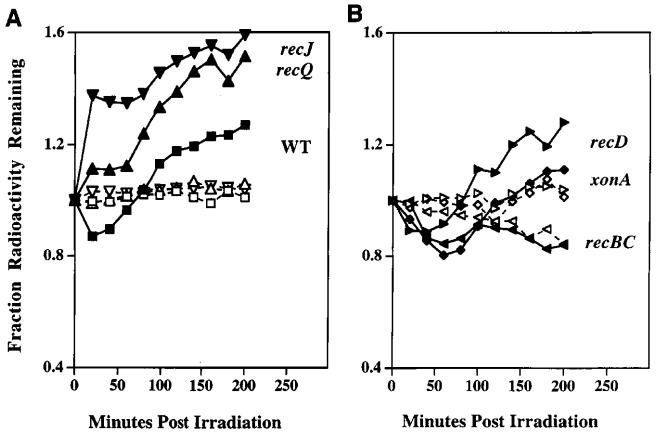

Fig. 2A, B Degradation of the nascent DNA does not occur in *recQ* or *recJ* mutants. [^{3}H]Thymidine was added to cells prelabeled with [^{14}C]thymine 10 s before the cells were placed in nonradiolabeled medium and irradiated with 25 J/m^2. The relative amount of radioactivity remaining in the DNA is plotted over time. *Open symbols*, ^{14}C-labeled (genomic) DNA; *filled symbols*, ^{3}H-labeled nascent DNA at the growing fork (labeled on graph). **A** Squares, parental cells; *triangles*, *recJ*; *inverted triangles*, *recQ*. **B** *Squares*, parental cells; *leftward arrowheads*, *recBC*; rightward arrowheads, *recD*; diamonds, *xonA*

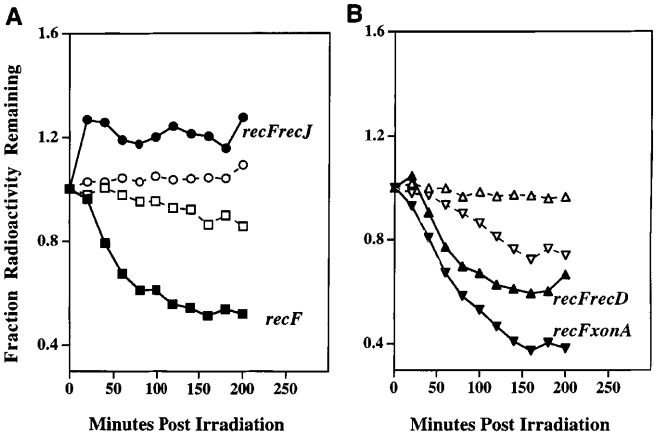

Fig. 3A, B The extensive degradation of nascent DNA seen in *recF* mutants does not occur in the absence of *recJ*. [^{3}H]Thymidine was added to cells prelabeled with [^{14}C]thymine 10 s before the cells were placed in nonradiolabeled medium and irradiated with 25 J/m^2. The relative amount of radioactivity remaining in the DNA is plotted over time. *Open symbols*, ^{14}C-labeled (genomic) DNA; *filled symbols*, ^{3}H-labeled nascent DNA at the growing fork (labeled on graph). **A** *Squares*, *recF*; *circles*, *recF recJ*. **B** Squares, *recF*; *triangles*, *recF recD*; *inverted triangles*, *recF xonA*

strand of the nascent DNA. To test this idea, we utilized radiolabeled riboprobes corresponding to the leading and lagging strand of replication in the *lacZ* gene. The leading and lagging strand riboprobes were hybridized to DNA which was isolated from irradiated *recF* mutants at various times after UV irradiation. However, because the nascent strand degradation represents such a limited amount of the total genomic DNA, we partially enriched our preparations for the nascent DNA by pulse labeling the *recF* mutants with [^{3}H]5-bromodeoxyuridine. DNA containing bromouracil instead of thymine has a higher buoyant density and can be isolated in isopycnic alkali CsCl gradients. While we could not achieve a complete separation of nascent DNA from bulk genomic DNA due to the short pulse-labeling times, we were able to discard a large portion of the genomic DNA, which would otherwise interfere with detection of a strand bias in the nascent DNA.

If the nascent DNA on both sides of the replication fork is degraded equally, the ratio between the lagging and leading strands of DNA should remain constant over time (as both strands are degraded). However, if degradation occurs preferentially on one strand, the ratio of the degraded strand relative to the nondegraded strand should decrease over time. When the isolated DNA was probed for the strands of the *lacZ* gene, we found that the lagging strand DNA decreased relative to the leading strand over time, consistent with the degradation occurring preferentially on the lagging strand of the nascent DNA (Fig. 4).

When the analysis was repeated using a *recF recJ* mutant, that bias in degradation was no longer observed and the ratio between the strands either remained constant or increased, also consistent with the idea that RecJ is degrading the lagging strand of the nascent DNA (Fig. 4). However, we cannot rule out the possibilities that the degradation within the *lacZ* gene is not representative of the rest of the genome or that the incorporation of 5-bromouracil, which is toxic to *E. coli*, somehow alters the normal degradation pattern.

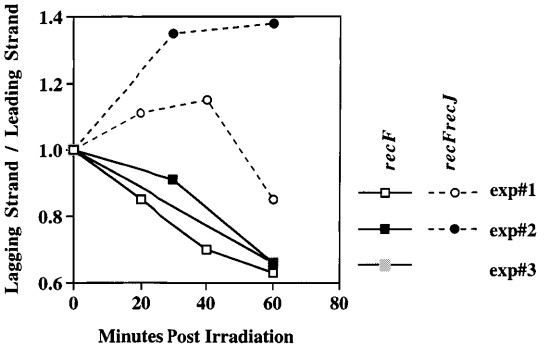

Fig. 4 The nascent lagging strand of the *lacZ* gene is preferentially degraded following UV irradiation in *recF* mutants but not *recF recJ* mutants. DNA enriched for the nascent DNA was isolated at various times after irradiating cells with 25 J/m^2 of UV. The DNA isolated at each time point was hybridized with riboprobes corresponding to either the leading or lagging strand of the *lacZ* gene and the amount of hybridization in each case was quantified. The ratio of the lagging strand signal to the leading strand signal at each time point is plotted. *Squares*, *recF* from three independent experiments; *circles*, *recFrecJ* from two independent experiments

Discussion

We have shown that RecJ and RecQ process the nascent DNA prior to the resumption of replication in UV-irradiated *E. coli*. The observations that the degradation preferentially occurs on the nascent lagging strand in the *lacZ* gene and that only half of the total nascent DNA is susceptible to degradation, support the idea that the nascent lagging strand is partially degraded by RecQ and RecJ in UV-irradiated *E. coli*.

The degradation of the lagging strand is consistent with the known polarities of RecQ and RecJ. The nascent DNA of a disrupted replication fork would be expected to terminate with a 3′ end on the leading strand and a 5′ end on the lagging strand (Fig. 5). RecJ, a 5′→3′ single strand-specific exonuclease, and RecQ, a helicase which translocates in the 3′→5′ direction when bound to single-stranded DNA, would therefore be expected to displace and degrade the nascent lagging strand. Interestingly, single strand binding protein (SSB), which has been shown to stimulate unwinding of DNA by RecQ, is thought to be present on the single-stranded region of the lagging strand template and may help target RecQ in vivo (Fig. 5)

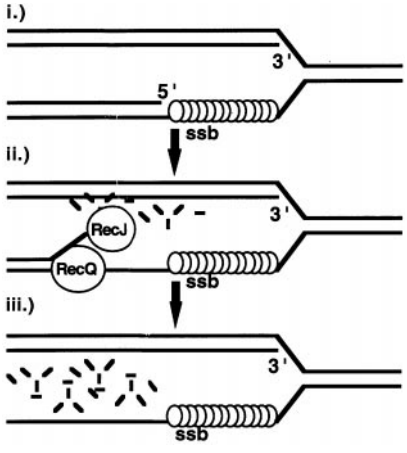

Fig. 5 Proposed model for the degradation of nascent DNA by RecJ and RecQ. During semiconservative replication, concurrent DNA synthesis in a 5′→3′ direction on both strands of the DNA template creates a single-stranded region on the lagging-strand template. The single-stranded region is thought to be coated with SSB and will vary in length depending upon the placement of the previous lagging-strand primer. Following the premature disruption of replication, 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 (i). RecQ, upon binding to the single-stranded region, translocates in a 5′→3′ direction, displacing the nascent lagging strand and making it susceptible to degradation by RecJ, a 5′ single-stranded exonuclease (ii). The processing creates an extended single-stranded region on the lagging strand template at the site of the disrupted replication fork (iii)

(Umezu and Nakayama 1993). The lack of degradation of nascent DNA in either a *recQ* or *recJ* mutant suggests a functional interaction between these gene products, in which RecQ is first required to unwind the nascent DNA before it can be degraded by RecJ. The observation that mutations in either *recQ* or *recJ* abrogate the replication fork processing also implies that other nucleases or helicases cannot substitute for them in this process.

We have shown that mutations in either *recBC* or *recD* do not prevent the processing of nascent DNA. Furthermore, these mutants, unlike *recA* or *recF,* have also been shown to recover replication following UV damage (Khidhir et al. 1985; Courcelle et al. 1997). These results are interesting because double-strand breaks have been shown to arise at high frequencies in the genomes of some DNA replication mutants (Michel et al. 1997; Seigneur et al. 1998). Based upon these observations, it has been hypothesized that replication may frequently "collapse" or "disintegrate" upon encountering DNA damage, producing double-strand breaks which are then repaired through a RecBCD recombination pathway (Bierne and Michel 1994; Kuzminov 1995a, 1995b; Michel et al. 1997; Seigneur et al. 1998). Yet, these observations, together with our previous studies, imply that, at least for UV-induced DNA damage, replication does not normally recover through a *recBCD*-dependent pathway. We would infer that in cells with fully functional replication machinery, double-strand breaks may not arise as frequently as has been hypothesized to occur during normal replication. However, exactly why *recBC* mutants exhibit such poor viability in the presence or absence of DNA damage is an important question which requires further study.

The observation that *xonA* mutants (ExoI) do not prevent the processing is interesting, in that if degradation also occurred on the nascent leading strand, the properties of ExoI would make it a likely candidate. *xonA* encodes a 3′→5′ exonuclease (opposite in polarity to that encoded by *recJ*) that copurifies with both RecA and SSB (Lehman and Nussbaum 1964; Molineaux et al. 1975; Phillips et al. 1988; Bedale et al. 1991, 1993). The lack of effect in *xonA* mutants could suggest that either the leading strand remains protected or that this assay may not relate to the cellular function of *exoI*.

The altered sites and frequencies at which recombination occurs in *recQ* and *recJ* mutants could suggest that the processing of nascent DNA helps to suppress chromosomal rearrangements or exchanges when replication is blocked by DNA damage (Ukita and Ikeda 1996; Hanada et al. 1997). One possible way how this could occur is that, when replication is blocked, partial degradation of the nascent lagging strand lengthens the triple-stranded substrate for RecA protein filaments to polymerize upon at the replication fork (Fig. 6). By maintaining the pairing of the replication fork strands, RecA may prevent the recombinagenic 3′ end of the nascent leading strand from "wandering", i.e. potentially pairing at other sites which might share some limited homology.

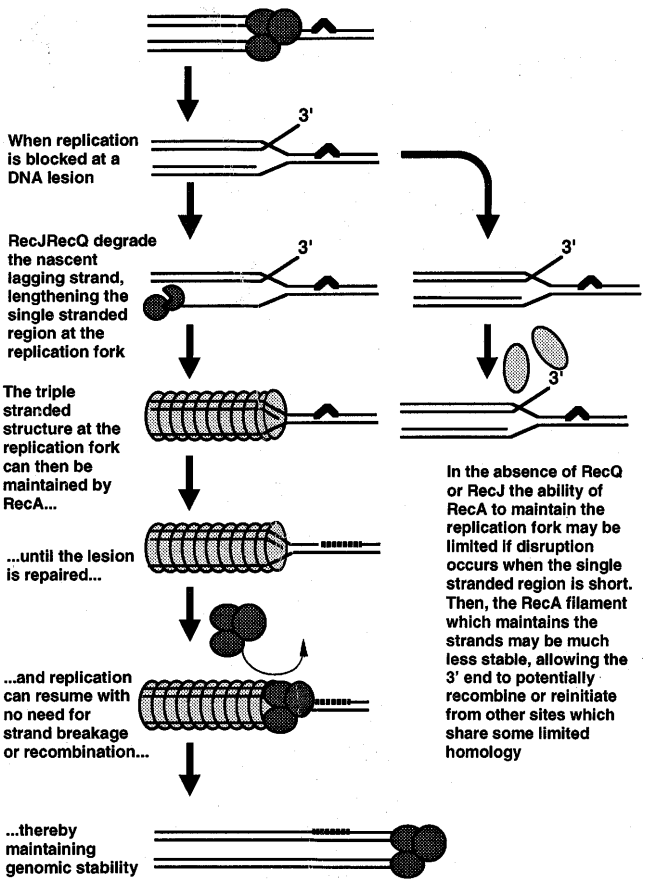

Fig. 6 Model for suppression of recombination by RecQ and RecJ

Most studies on RecA-mediated homologous strand pairing have focused primarily upon its role in catalyzing recombination events. However, as discussed in the Introduction, during genomic replication, the presence of RecA is absolutely required to maintain replication forks when they are blocked by DNA damage (Horii and Suzuki 1968; Howard-Flanders 1968). Purified RecA binds single-stranded DNA and progressively pairs it with homologous duplex DNA in a 5′→3′ direction to form a triple-stranded structure (for reviews see Smith 1988; Kowalczykowski et al. 1994; Roca and Cox 1997). During recombination, this activity is thought to be critical in bringing together homologous pieces of DNA. During the processive, semiconservative replication of the genome, however, this same activity could also be expected to play a critical role. To understand how the biochemical activity of RecA may operate to stabilize replication forks, consider that the template of an ongoing replication fork contains one strand which has not yet been replicated (single-stranded DNA) and one strand which has been replicated (duplex DNA that is homologous to the single-stranded region) as shown in Fig. 5. Given these substrates, RecA would be expected to maintain a joint molecule between the replicated template strand and the nonreplicated template. The rapid degradation of the genomic DNA which occurs when replication encounters a DNA lesion in

recA mutants suggests that this pairing is required to protect the replication fork from degradation. Importantly however, maintaining this pairing would also preserve the accurate and semiconservative duplication of the template once replication could again resume (i.e. after the blocking lesion has been repaired).

The role of RecJ and RecQ in processing disrupted replication forks is suggestive when one considers their function in assays which score for recombinational events. To catalyze recombinational events, RecJ and RecQ are thought to function by creating single-stranded DNA extensions for RecA-mediated homologous strand pairing (Kowalczykowski et al. 1994; Harmon and Kowalczykowski 1998). During genomic replication, the DNA substrate processed by RecJ and RecQ is at the blocked replication fork, suggesting that by creating single-stranded DNA at this site, they promote homologous pairing between the strands of the replication fork. In a sense, no substrate could be a more legitimate site for homologous strand pairing than the original site of blockage! This is the only site in the cell at which homologous strand pairing would maintain the semiconservative replication of the genome.

RecQ belongs to a highly conserved family of helicases which has been shown to be important for maintaining genomic integrity and semiconservative DNA replication. The genes defective in Bloom and Werner syndromes, rare genetic disorders in humans, encode DNA helicases which share large portions of homology with the *E. coli* RecQ protein (Ellis et al. 1995; Yu et al. 1996). At the cellular level, these disorders are associated with high rates of sister chromatid exchanges, chromosomal rearrangements, and a general genomic instability (Fukuchi et al. 1989; Langlois et al. 1989; Monnat et al. 1992). Bloom syndrome cells are hypersensitive to UV and exhibit abnormal replication patterns following DNA damage (Giannelli et al. 1977; Lonn et al. 1990). Cells from Werner syndrome patients display prolonged or abnormal replication during S-phase (Hanaoka et al. 1985). Patients with Bloom syndrome exhibit growth retardation, decreased fertility, immune deficiencies, and an increased incidence of cancer (German 1995). Werner syndrome patients also exhibit growth retardation, decreased fertility, and cancer predisposition but additionally exhibit characteristics of accelerated or premature aging (Epstein et al. 1966).

RecQ homologs in other organisms also have phenotypes associated with maintaining semiconservative DNA replication. In *S. pombe*, Rqh-1 is required to suppress recombination which leads to an irreversible S-phase arrest (Stewart et al. 1997). In *S. cerevisiae*, mutations in *SGS1*, which encodes a RecQ homolog that interacts with topoisomerases II and III, are associated with increased rates of chromosome nondisjunction (Gangloff et al. 1994; Watt et al. 1995, 1996).

At the level of the chromosome, the frequency of strand exchange during genomic replication correlates directly with cell death, genomic instability, and, in higher organisms, a predisposition to cancer. This observation implies that there is a major conceptual difference between a protein which is required for recombination to occur and a recombination protein. Many proteins have been isolated because they affect recombination frequencies. Quite naturally, these proteins have been characterized primarily for their ability to rearrange DNA. During an asexual reproductive cycle however, many of these proteins, including the *E. coli* RecA protein, are intimately associated with the ability of cells to carry out the replication of the genome. The fact that genomic replication is semiconservative suggests that these proteins may help maintain this process. Recombination events may often represent the products of tolerated, but inappropriate, resolution of strand-pairing events. Understanding the circumstances which can lead to these strand exchanges and rearrangements, however, is critical to understanding how genomic stability is maintained. Both RecJ and RecQ were identified as proteins which were required for recombination to occur under certain conditions (Lovett and Clark 1984; Nakayama et al. 1984). However, on the chromosome, RecJ and RecQ appear to function at the replication fork. That the cellular target of RecJ and RecQ in *E. coli* is the nascent DNA of blocked replication forks suggests that their role in maintaining genomic stability could be linked to their ability to maintain the replication fork during *rec*overy rather than *rec*ombination.

Acknowledgements We thank Ann Ganesan, Charles Allen Smith, and Jennifer Halliday for their questions, comments, and critical reading of the manuscript. We also appreciate the helpful suggestions from the referees of this manuscript. This research is supported by grant CA44349 from the National Cancer Institute. JC is supported in part by a traineeship from the National Cancer Institute (DHHS no. CA09302).

References

Bedale WA, Inman RB, Cox MM (1991) RecA protein-facilitated DNA strand breaks. A mechanism for bypassing DNA structural barriers during strand exchange. J Biol Chem 266:6499–6510

Bedale WA, Inman RB, Cox MM (1993) A reverse DNA strand exchange mediated by recA protein and exonuclease I. J Biol Chem 268:15004–15016

Bierne H, Michel B (1994) When replication forks stop. Mol Microbiol 13:17–23

Clark AJ, Chamberlin M (1966) Abnormal metabolic response to ultraviolet light of a recombination deficient mutant of *Escherichia coli* K12. J Mol Biol 19:442–454

Clark AJ, Margulies AD (1965) Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12. Proc Natl Acad Sci USA 53:451–459

Courcelle J, Carswell-Crumpton C, Hanawalt PC (1997) *recF* and *recR* are required for the resumption of replication at DNA replication forks in *Escherichia coli*. Proc Natl Acad Sci USA 94:3714–3719

Courcelle J, Crowley DJ, Hanawalt PC (1998) Recovery of DNA replication in UV-irradiated *Escherichia coli* requires both excision repair and RecF protein function. J Bacteriol 181:916–922

Ellis NA, Groden J, Ye TZ, Straughen J, Lennon DJ, Ciocci S, Proytcheva M, German J (1995) The Bloom's syndrome gene product is homologous to RecQ helicases. Cell 83:655–666

Epstein CJ, Martin GM, Schultz AL, Motulsky AG (1966) Werner's syndrome: a review of its symptomology, natural history,

pathologic features, genetics, and relationship to the natural aging process. Medicine 45:177–221
Fukuchi K, Martin GM, Monnat RJ Jr (1989) Mutator phenotype of Werner syndrome is characterized by extensive deletions [Erratum appears in Proc Natl Acad Sci USA 1989 Oct; 86:7994]. Proc Natl Acad Sci USA 86:5893–58937
Ganesan AK (1974) Persistence of pyrimidine dimers during post-replication repair in ultraviolet light-irradiated *Escherichia coli*. J Mol Biol 87:103–119
Ganesan AK, Smith KC (1971) The duration of recovery and repair in excision-deficient derivatives of *Escherichia coli* K-12 after ultraviolet light irradiation. Mol Gen Genet 113:285–296
Gangloff S, McDonald JP, Bendixen C, Arthur L, Rothstein R (1994) The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. Mol Cell Biol 14:8391–8398
German J (1995) Bloom's syndrome. In: Cohen PR, Kurzrock R (eds) Dermatologic clinics. WB Saunders, Philadelphia, pp 7–18
Giannelli F, Benson PF, Pawsey SA, Polani PE (1977) Ultraviolet light sensitivity and delayed DNA-chain maturation in Bloom's syndrome fibroblasts. Nature 265:466–469
Hanada K, Ukita T, Kohno Y, Saito K, Kato J, Ikeda H (1997) RecQ DNA helicase is a suppressor of illegitimate recombination in *Escherichia coli*. Proc Natl Acad Sci USA 94:3860–3865
Hanaoka F, Yamada M, Takeuchi F, Goto M, Miyamoto T, Hori T (1985) Autoradiographic studies of DNA replication in Werner's syndrome cells. Adv Exp Med Biol 190:439–457
Hanawalt P, Brempelis I (1967) Selective degradation of newly-replicated DNA after inhibition of DNA synthesis in *Escherichia coli*. Proceedings of the 7th International Congress of Biochemistry, Tokyo, p 650
Harmon FG, Kowalczykowski SC (1998) RecQ helicase, in concert with RecA and SSB proteins, initiates and disrupts DNA recombination. Genes Dev 12:1134–1144
Horii Z, Clark AJ (1973) Genetic analysis of the *recF* pathway to genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. J Mol Biol 80:327–344
Horii Z, Suzuki K (1968) Degradation of the DNA of *Escherichia coli* K12 rec-(JC1569b) after irradiation with ultraviolet light. Photochem Photobiol 8:93–105
Howard-Flanders P (1968) Genes that control DNA repair and genetic recombination in *Escherichia coli*. Adv Biol Med Phys 12:299–317
Howard-Flanders P, Theriot L, Stedeford JB (1969) Some properties of excision-defective recombination-deficient mutants of *Escherichia coli* K-12. J Bacteriol 97:1134–1141
Khidhir MA, Casaregola S, Holland IB (1985) Mechanism of transient inhibition of DNA synthesis in ultraviolet-irradiated *E. coli*: inhibition is independent of *recA* whilst recovery requires RecA protein itself and an additional, inducible SOS function. Mol Gen Genet 199:133–140
Kolodner R, Fishel RA, Howard M (1985) Genetic recombination of bacterial plasmid DNA: effect of RecF pathway mutations on plasmid recombination in *Escherichia coli*. J Bacteriol 163:1060–1066
Kowalczykowski SC, Dixon DA, Eggleston AK, Lauder SD, Rehrauer WM (1994) Biochemistry of homologous recombination in *Escherichia coli*. Microbiol Rev 58:401–465
Kuzminov A (1995) Collapse and repair of replication forks in *Escherichia coli*. Mol Microbiol 16:373–384
Kuzminov A (1995) Instability of inhibited replication forks in *E. coli*. Bioessays 17:733–741
Langlois RG, Bigbee WL, Jensen RH, German J (1989) Evidence for increased in vivo mutation and somatic recombination in Bloom's syndrome. Proc Natl Acad Sci USA 86: 670–674
Lehman IR, Nussbaum AL (1964) The deoxyribonucleases of *Escherichia coli*. V. On the specificity of exonuclease I (phosphodiesterase). J Biol Chem 239:2628–2636
Lonn U, Lonn S, Nylen U, Winblad G, German J (1990) An abnormal profile of DNA replication intermediates in Bloom's syndrome. Cancer Res 50:3141–3145
Lovett ST, Clark AJ (1984) Genetic analysis of the *recJ* gene of *Escherichia coli* K-12. J Bacteriol 157:190–196
Lovett ST, Kolodner RD (1989) Identification and purification of a single-stranded-DNA-specific exonuclease encoded by the *recJ* gene of *Escherichia coli*. Proc Natl Acad Sci USA 86:2627–2631
Mahdi AA, Lloyd RG (1989) Identification of the *recR* locus of *Escherichia coli* K-12 and analysis of its role in recombination and DNA repair. Mol Gen Genet 216:503–510
Mellon I, Hanawalt PC (1989) Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. Nature 342: 95–98
Michel B, Ehrlich SD, Uzest M (1997) DNA double-strand breaks caused by replication arrest. EMBO J 16:430–438
Molineaux IJ, Pauli A, Gefter ML (1975) Properties of the *Escherichia coli* DNA-binding (unwinding) protein. Interaction with nucleolytic enzymes and DNA. J Mol Biol 98:811–825
Monnat RJ Jr, Hackmann AF, Chiaverotti TA (1992) Nucleotide sequence analysis of human hypoxanthine phosphoribosyltransferase (*HPRT*) gene deletions. Genomics 13:777–787
Nakayama H, Nakayama K, Nakayama R, Irino N, Nakayama Y, Hanawalt PC (1984) Isolation and genetic characterization of a thymineless death-resistant mutant of *Escherichia coli* K12: identification of a new mutation (*recQ1*) that blocks the RecF recombination pathway. Mol Gen Genet 195:474–480
Nakayama K, Irino N, Nakayama H (1985) The *recQ* gene of *Escherichia coli* K12: molecular cloning and isolation of insertion mutants. Mol Gen Genet 200:266–271
Phillips GJ, Prasher DC, Kushner SR (1988) Physical and biochemical characterization of cloned *sbcB* and *xonA* mutations from *Escherichia coli* K-12. J Bacteriol 170:2089–2094
Roca AI, Cox MM (1997) RecA protein: structure, function, and role in recombinational repair. Prog Nucleic Acid Res Mol Biol 56:129–223
Rothman RH (1978) Dimer excision and repair replication patch size in a *recL152* mutant of *Escherichia coli* K-12. J Bacteriol 136:444–448
Rupp WD, Howard-Flanders P (1968) Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J Mol Biol 31:291–304
Rupp WD, Wilde CEI, Reno DL, Howard-Flanders P (1971) Exchanges between DNA strands in ultraviolet-irradiated *Escherichia coli*. J Mol Biol 61:25–44
Seigneur M, Bidnenko V, Ehrlich SD, Michel B (1998) RuvAB acts at arrested replication forks. Cell 95:419–430
Setlow RB, Carrier WL (1964) The disappearance of thymine dimers from DNA: an error-correcting mechanism. Proc Natl Acad Sci USA 51: 226–231
Setlow RB, Swenson PA, Carrier WL (1963) Thymine dimers and inhibition of DNA synthesis by ultraviolet irradiation of cells. Science 142:1464–1466
Smith CA, Cooper PK, Hanawalt PC (1981) Measurement of repair replication by equilibrium sedimentation. In: Friedberg EC, Hanawalt PC (eds) DNA Repair: a manual of research procedures. Marcel Dekker, New York, pp 289–305
Smith GR (1988) Homologous recombination in procaryotes [published erratum appears in Microbiol Rev 52:304]. Microbiol Rev 52:1–28
Stewart E, Chapman CR, Al-Khodairy F, Carr AM, Enoch T (1997) *rqh1+*, a fission yeast gene related to the Bloom's and Werner's syndrome genes, is required for reversible S phase arrest. EMBO J 16:2682–2692
Templin A, Kushner SR, Clark AJ (1972) Genetic analysis of mutations indirectly suppressing *recB* and *recC* mutations. Genetics 72:205–215
Ukita T, Ikeda H (1996) Role of the *recJ* gene product in UV-induced illegitimate recombination at the hotspot. J Bacteriol 178: 2362–2367
Umezu K, Nakayama H (1993) RecQ DNA helicase of *Escherichia coli*. Characterization of the helix-unwinding activity with emphasis on the effect of single-stranded DNA-binding protein. J Mol Biol 230:1145–1150

Watt PM, Hickson ID, Borts RH, Louis EJ (1996) *SGS1*, a homologue of the Bloom's and Werner's syndrome genes, is required for maintenance of genome stability in *Saccharomyces cerevisiae*. Genetics 144:935–945

Watt PM, Louis EJ, Borts RH, Hickson ID (1995) Sgs1: a eukaryotic homolog of *E. coli* RecQ that interacts with topoisomerase II in vivo and is required for faithful chromosome segregation. Cell 81:253–260

Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, Alisch R, Matthews S, Nakura J, Miki T, Ouais S, Martin GM, Mulligan J, Schellenberg GD (1996) Positional cloning of the Werner's syndrome gene. Science 272:258–262