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A model for the *recA*-dependent repair of excision gaps in UV-irradiated *Escherichia coli*

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Summary

We have tested and supported the hypothesis that, in UV-irradiated *Escherichia coli, recA*-dependent nucleotide excision repair only functions in the replicated portion of the chromosome (i.e., where sister duplexes exist). Using a *dnaA*(Ts) mutation to align the chromosomes (i.e., all rounds of DNA replication were completed, and new rounds could not be initiated), we studied the genetic control of excision repair (measured as the repair of excision gaps in DNA) in cells with unreplicated chromosomes, and also in cells with partially replicated chromosomes. The excision repair that occurred in cells with unreplicated chromosomes was *recA* independent, but the excision repair that occurred in cells with partially replicated chromosomes was partially *recA* dependent. We found no evidence of interchromosomal recombination in *recA*-dependent excision repair. The majority of this *recA*-dependent excision repair was *recF* dependent, and a small portion was *recB* dependent. The *recF* and *recB* genes are suggested to function in excision repair in a manner similar to their function in postreplication repair, i.e., in the replicated portion of a chromosome, the RecF pathway repairs gaps, and the RecB pathway repairs the DNA double-strand breaks that arise at unrepaired gaps.

Studies on the formation and repair of excision gaps (assayed with alkaline sucrose gradients) in the DNA of various DNA-repair-deficient mutants of *Escherichia coli* have demonstrated that there are growth-medium-dependent and growth-medium-independent pathways of nucleotide excision repair (Youngs et al., 1974). The majority of these excision gaps are repaired by the growth-medium-independent, *recA*-independent pathway that requires DNA polymerase I, while the growth-medium-dependent repair of excision gaps is dependent upon functional *recA, recB,* and *lexA* genes, and is inhibited by chloramphenicol (Youngs et al., 1974), suggesting that at least a portion of this repair pathway is inducible. A functional *recF* gene is also required for the repair of excision gaps in growth medium (present study).

Two major pathways of *recA*-dependent postreplication repair have been described. The *recF*-dependent pathway is largely responsible for the repair of DNA daughter-strand gaps (Ganesan and Seawell, 1975; Kato, 1977; Rothman and Clark, 1977; Wang and Smith, 1983). The *recB*-dependent pathway plays little role in the repair

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of these gaps, but is required for the repair of DNA double-strand breaks that arise at unrepaired DNA daughter-strand gaps (Wang and Smith, 1983, 1986).

Because growth-medium-dependent nucleotide-excision repair and postreplication repair both require functional *recA, recB* and *recF* genes, we suggest that the recombinational processes that function in postreplication repair (e.g., Wang and Smith, 1983) may also function in the *recA*-dependent repair of excision gaps.

Lesions produced in DNA by UV irradiation can be assumed to be distributed in both the unreplicated (i.e., where no sister duplexes exist) and replicated (i.e., where sister duplexes exist) regions of an *E. coli* chromosome. However, the lesions in these two 'domains' may have different consequences as regards DNA repair. For example, lesions in the unreplicated domain probably can only be repaired by the *recA*-independent pathway of excision repair, i.e., recombinational processes are unlikely because there are no intrachromosomal sister duplexes present. In the replicated domain, however, intrachromosomal sister duplexes are present, and both *recA*-dependent and *recA*-independent repair processes are possible.

We have used a *dnaA*(Ts) mutation to control the amount of replicated DNA in cells, and have used this procedure to explore the mechanism of *recA* involvement in excision repair. We find that *recA*-dependent excision repair occurs in the replicated portion of the chromosome, but not in the unreplicated portion of the chromosome. Interchromosomal recombination does not appear to play a role in *recA*-dependent excision repair. Furthermore, the *recF*-dependent and *recB*-dependent processes for excision repair are suggested to be similar to the two major processes for postreplication repair (Wang and Smith, 1983), i.e., in the replicated portion of a chromosome, the RecF pathway repairs gaps, and the RecB pathway repairs the DNA double-strand breaks that arise at unrepaired gaps.

Materials and methods

Bacterial strains, media and cultures

The strains of *E. coli* used in this study are listed in Table 1. Transduction was carried out by the method described by Miller (1972). All strains were checked for bacteriophage P1 lysogeny.

The salts buffer (DTM) has been described (Sharma and Smith, 1985). The minimal growth medium (MM) was DTM supplemented with 0.4% glucose, thymine (10 μg/ml for overnight cultures, and 2 μg/ml for logarithmic-phase cultures), 0.5 μg/ml thiamine hydrochloride, and each required amino acid at 1 mM. Minimal plating medium was MM solidified with 1.6% (w/v) Difco Noble agar, which does not inhibit the growth-medium-dependent pathway of excision repair (Van der Schueren et al., 1974). Phosphate buffer (PB) was Na_2HPO_4 (5.83 g/l) and KH_2PO_4 (3.53 g/l), pH 7.0.

Logarithmic-phase cultures were obtained by diluting (100-fold) a fresh overnight culture into homologous MM (thymine at 2 μg/ml), and growing by shaking at 37°C or at 30°C (for temperature-sensitive mutants) to an optical density at 650 nm (OD_{650}) of 0.4 ($\sim 3 \times 10^8$ cells/ml, Zeiss PMQII spectrophotometer). Cells were harvested by filtration (HAWG, 0.45-μm pore size; Millipore Corp.), washed with MM, and resuspended in MM at an OD_{650} of 0.1.

UV irradiation

The UV-irradiation (254 nm) conditions have been described (Sharma and Smith, 1983). For viability measurements, irradiated and control cell suspensions were diluted in PB and plated on MM. Incubation was for 2–3 days at 30°C. All experiments were done under yellow lights to prevent photoreactivation.

Measurement of DNA repair

The cells were prelabeled by growing them for at least 4 generations in MM containing thymine at 2 μg/ml and [*Me*-^{3}H]thymine at 20 μCi/ml (57 Ci/mmole, Amersham Corp.) at the temperature appropriate for their growth. Additional experimental details specific for each strain used are given in Results.

After UV irradiation and 120 min of repair incubation at the appropriate temperature (to allow the formation and maximal repair of excision gaps; data not shown), the cells were converted to spheroplasts by mixing 0.2 ml of cells with 0.2 ml

TABLE 1

LIST OF STRAINS OF *E. coli* [a]

Strain No.	Relevant genotype	Other characteristics	Source, derivation, or reference
SR248	+	*leuB19 metE70 thyA36 deo(C2?) bioA2 lacZ53 malB45 rha-5 rpsL151*	R.B. Helling
SR669	*recA56*	HfrKL16 *ilv-318 thr-300 srlA300::*Tn*10 rpsE300*	A.J. Clark (JC10240)
SR923	*recA56*	Same as SR248, except Thy$^+$	Sargentini and Smith (1981)
SR1105	*recF143*	Same as SR248, except *tnaA300::*Tn*10*	Sargentini and Smith (1983)
SR1164	*recB21*	Same as SR248, except Thy$^+$	Sargentini and Smith (1983)
SR1450	*recA56*	Same as SR923, except *thyA*	SR923, spontaneous Tmp^r selection
SR1451	*recB21*	Same as SR1164, except *thyA*	SR1164, spontaneous Tmp^r selection
SR1572	*dnaB279*	*leu thr thyA deo thi lac tonA rpsL* suII$^+$	A. Kornberg (E279)
SR1603	*dnaA5*	*arg-28 his-47 leu-19 met-55 pro-19 trp-25 thyA59 deoB23 lac-11 gal-11 rpsL56 sul-1 hsdS* (K-12)	A. Kornberg (WM493)
SR1604	*dnaA5 recA56*	Same as SR1603, except *srlA300::*Tn*10*	P1::Tn*9*cts·SR669×SR1603; Tc^r selection
SR1651	*dnaB279 recA56*	Same as SR1572, except *srlA300::*Tn*10*	SR1572×P1::Tn*9*ts·SR669, Tc^r selection

[a] Genotype symbols are those used by Bachmann (1983). All strains are F$^-$ (except strain SR669) and λ^-. Strains SR1603 and SR1604 are B strains, all others are K-12. Tc^r and Tmp^r mean that cells became resistant to tetracycline and trimethoprim, respectively.

of ice-cold lysozyme solution (400 μg/ml in 0.07 M Tris–0.017 M EDTA, pH 7.6). After 10–15 min on ice, 0.1 ml of the spheroplast suspension was layered on top of linear gradients (4.8 ml of 5–20% sucrose in 0.1 N NaOH and 0.1% (v/v) Triton X-100, capped with 0.1 ml of 0.5 N NaOH). The layered gradients were held for ~ 90 min in the dark at room temperature, and then were centrifuged in Beckman/Spinco SW50.1 rotors at 10 000 rpm for 16 h at 20°C, unless otherwise mentioned. [^{14}C]Thymine-labeled bacteriophage T2 DNA was used as a molecular weight marker. The methods for processing the gradients (Hamelin et al., 1976) and calculating the data (Youngs and Smith, 1976a, b) have been described. A value of 2.8×10^9 dalton was used for the molecular weight of the *E. coli* genome (Cairns, 1963).

Measurement of DNA content per cell

DNA content was measured by the diphenylamine method (Burton, 1956) as described by Friesen (1968). Briefly, cell numbers were determined in a Coulter Counter. Aliquots of cells (~ 10^9 cells) were precipitated with cold 5% trichloroacetic acid (TCA), and the precipitations were collected by centrifugation at 4°C and washed twice with cold 5% TCA. The TCA precipitates were resuspended in 0.25 ml of 1 M perchloric acid, heated in a water bath for 10 min at 70°C, and left overnight at room temperature. The samples were centrifuged at room temperature, supernatants were removed and saved, and the precipitates were extracted once again with 0.25 ml of 1 M perchloric acid. The two supernatants were pooled (total volume 0.5 ml), mixed with 1 ml of freshly prepared diphenylamine reagent, and incubated for 16 h at 30°C before measuring the absorbance at 600 nm. Deoxyadenosine was used as the standard.

Results

One prediction from our hypothesis for *recA*-dependent excision repair in *E. coli* is that if cells are arrested in DNA synthesis after completing all rounds of replication (i.e., so that no intrachromosomal sister duplexes are present) and are then UV irradiated, the repair of excision gaps in

DNA should be *recA* independent. That is, recombination cannot occur in the absence of homologous genetic material. To test this prediction we have employed a temperature-sensitive *dnaA* mutant of *E. coli* (Tippe-Schindler et al., 1979). The *dnaA* mutation prevents the initiation of new rounds of chromosome replication at the nonpermissive temperature, but does not affect DNA chain elongation at existing replication forks (Hirota et al., 1970; Zahn et al., 1977). When *dnaA* and *dnaA recA* cells growing at 30°C were shifted to 42.5°C, DNA synthesis was completely arrested in the cultures by 120 min (data not shown), in agreement with previous data (Zahn et al., 1977).

To assay for the repair of excision gaps, *dnaA* cells were prelabeled by growing them at 30°C in MM containing [^{3}H]thymine for 4 or more generations, and then they were shifted to 42.5°C for 180 min to align their chromosomes (i.e., all rounds of DNA replication were completed, and new rounds could not be initiated). The cells were UV irradiated at 42.5°C, and further incubated at 42.5°C for 120 min to allow for the formation of excision gaps (data not shown), and for their maximal repair (Fig. 1). Fig. 1 (open symbols) shows the number of unrepaired DNA single-strand breaks in the *dnaA* and *dnaA recA* cells after the completion of repair at 42.5°C. The presence of the *recA* mutation did not affect the repair of excision gaps when the chromosomes were aligned before UV irradiation, and kept aligned during repair incubation. Under these conditions, both the *dnaA* and *dnaA recA* strains repaired almost all of the excision gaps (Fig. 1, open symbols).

A second prediction from our hypothesis is that excision repair should become partially *recA* dependent if the cells contain intrachromosomal sister duplexes so that intrachromosomal recombination can occur. To test this prediction, *dnaA* cells with aligned chromosomes were shifted back to 30°C for 30 min, which allowed DNA replication to be initiated and the DNA content of the cells to increase about 1.4-fold (Table 2). At this time the cells were filter harvested, resuspended in nonradioactive MM, UV irradiated, and then allowed to repair at 30°C. Cells containing partially replicated chromosomes were more deficient in the repair of excision gaps (Fig. 1, closed symbols) than were cells whose chromosomes were aligned (Fig. 1, open symbols). The *recA* cells showed the greatest deficiency in the repair of excision gaps under these conditions (Fig. 1).

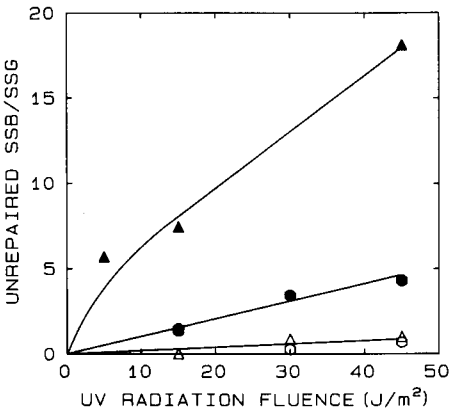

Fig. 1. Effect of a *recA* mutation on the repair of excision gaps in the DNA of *E. coli dnaA*(Ts) cells. After DNA labeling and chromosomal alignment at 42.5°C (see Materials and Methods), the cells were UV irradiated and then further incubated at 42.5°C for 120 min to allow the repair of excision gaps (○, △), or the cells were shifted to 30°C for 30 min to allow the cells to replicate about 40% of their DNA, and then were UV irradiated and allowed to repair for 120 min at 30°C (●, ▲). The yield of unrepaired DNA single-strand breaks (SSB) per single-strand genome (SSG) was determined as described in Materials and Methods. ○, ●, *dnaA5* (SR1603); △, ▲, *dnaA5 recA56* (SR1604). The data are the average of 2 Expts.

In order to test if the movement of the replication fork and the process of postreplication repair might affect our results on excision repair shown in Fig. 1 (closed symbols), we used *dnaB*(Ts) strains. DNA synthesis stops almost immediately in a *dnaB* strain when the temperature is shifted to 42°C (Wechsler and Gross, 1971). Shifting *dnaB* cells to 42°C after UV irradiation would block the formation of DNA daughter-strand gaps, which are the substrates for postreplication repair, thus allowing one to study excision repair in the absence of postreplication repair. We found no difference in the repair of excision gaps whether UV-irradiated *dnaB* cells were incubated at 42.5°C (i.e., in the absence of active replication forks) or at 30°C (i.e., in the presence of active replication forks) (Fig. 2, compare ■, □). (N.B.: Only the DNA synthesized prior to UV irradia-

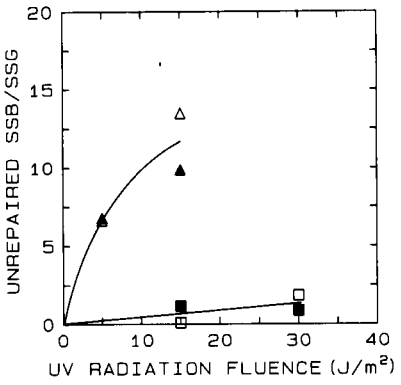

Fig. 2. The repair of excision gaps in the DNA of *E. coli* *dnaB*(Ts) cells. The cells were labeled by growing them at 30°C in MM containing [^{3}H]thymine for 4 or more generations, UV irradiated in MM, and then allowed to repair at 30°C (■, ▲) or 42.5°C (□, △), □, ■, *dnaB279* (SR1572); △, ▲ *dnaB279 recA56* (SR1651). The data are the average of 2 Expts.

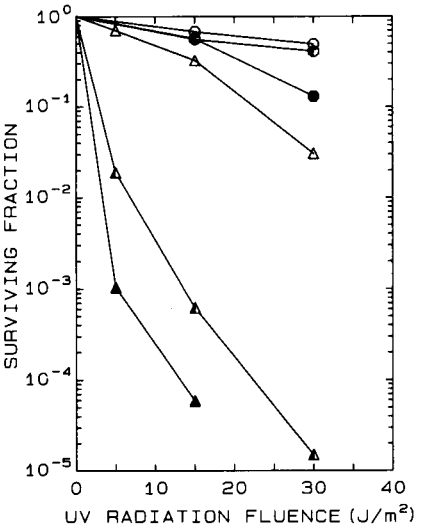

Fig. 3. UV-radiation survival of *dnaA*(Ts) and *dnaA*(Ts) *recA* cells of *E. coli*. Logarithmic-phase cells (30°C) were shifted to 42.5°C and incubated for 3 h to align the chromosomes. Cells were UV irradiated and plated immediately (◐, ◭), or after 120 min of repair incubation at 42.5°C (○, △). Another portion of cells was shifted to 30°C for 30 min to allow the cells to replicate about 40% of their DNA before they were UV irradiated (●, ▲). The plates were incubated at 30°C for 2–3 days before the survivors were scored. ◐, ○, ●, *dnaA5* (SR1603); ▲, △, ▲, *dnaA5 recA56* (SR1604). The data are the average of 2 Expts.

tion was labeled with [^{3}H]thymine and assayed in the experiments presented in this work.) Thus, an active replication fork and the formation and repair of DNA daughter-strand gaps neither interfere with nor are required for *recA*-dependent excision repair. However, the presence of a *recA* mutation inhibited the repair of excision gaps under conditions where cells had intrachromosomal sister duplexes whether in the presence or absence of active replication forks (Fig. 2).

UV-radiation survival was determined under the conditions used for the repair studies in order to determine if the observed repair reflects enhanced survival. The *dnaA recA* cells (chromosomes aligned) were very radiation sensitive if plated at 30°C immediately after UV irradiation, however, they became much more resistant to UV irradiation if they were incubated for 2 h at 42.5°C (i.e., where the reinitiation of DNA replication was prevented), to allow the completion of *recA*-independent excision repair, before being plated at 30°C (Fig. 3). Longer incubation times at 42.5°C (up to 4 h) did not result in any additional increase in survival (data not shown). The *dnaA* cells however, showed very little increase in survival from this treatment (Fig. 3). The *dnaA* and *dnaA recA* cells that had been allowed to reinitiate DNA synthesis and to increase their DNA content 1.4-fold were more radiation sensitive than when their chromosomes were aligned (Fig. 3).

We determined the DNA content per cell under our experimental conditions in order to evaluate whether our results for the lack of *recA*-dependent excision repair were due to the absence of sister duplexes or could be due to the absence of sister chromosomes. When *dnaA* cells (strain SR1603) were incubated for 3 h at 42.5°C to complete all rounds of DNA replication (i.e., the cells should contain no intrachromosomal sister duplexes), the cells contained an average of 1.73 genome equivalents of DNA per cell (Table 2), indicating that a major fraction of the cell population contained 2 or more chromosomes per cell. These results suggest that the lack of *recA*-dependent excision repair under these conditions was due to the absence of intrachromosomal sister duplexes, rather than to an absence of sister chromosomes.

TABLE 2

DNA CONTENT PER *dnaA* CELL [a]

Experimental condition	Average DNA/cell ($\times 10^{15}$ g)	Average genome equivalents [b]
(1) log phase at 30°C	5.73	1.23
(2) 3 h at 42.5°C	8.02	1.73
(3) 30 min at 30°C	11.66	2.51

[a] Data are the average of 2 independent Expts. (see Materials and Methods). The cells were shifted sequentially through the 3 experimental conditions; samples were taken for analysis at the end of each treatment.

[b] Using a value of 2.8×10^9 dalton for the molecular weight of the *E. coli* genome (Cairns, 1963), 1 genome equivalent is equal to 4.649×10^{-15} g of DNA.

The results in Fig. 1 suggest that *recA*-dependent excision repair only occurs in the replicated portion of the chromosome. Since excision gaps formed in the replicated portion of the chromosome appear to present a repair situation analogous to that for DNA daughter-strand gaps during postreplication repair, we tested whether genes that function in postreplication repair (e.g., Wang and Smith, 1983), also function in *recA*-dependent excision repair. We used logarithmic-phase cells, which, on average, are expected to have 44% of their DNA replicated (Maaløe and Hanawalt, 1961). When compared to wild-type cells, both the *recB* and *recF* cells were deficient in the repair of excision gaps, but not as deficient as *recA* cells (Fig. 4). These experiments could not be performed in the *dnaA* background, since the *recF* and *dnaA* mutations are separated by less than 0.1 min (Bachmann, 1983).

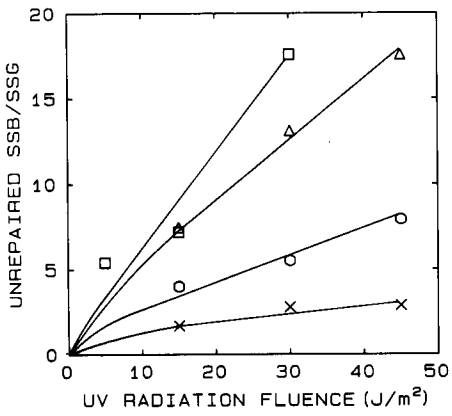

Fig. 4. Effect of *recB* and *recF* mutations on the repair of excision gaps in the DNA of *E. coli* cells in logarithmic-phase growth. The cells were grown at 37°C, UV irradiated in MM and then allowed to repair for 120 min at 37°C. The yield of unrepaired DNA single-strand breaks (SSB) per single-strand genome (SSG) was determined as described in Materials and Methods. X, wild type (SR248); O, *recB21* (SR1451); △, *recF143* (SR1105); □, *recA56* (SR1450). The data are the average of 2 Expts.

Discussion

We have postulated that the mechanisms for the *recA*-dependent repair of excision gaps in the DNA of UV-irradiated *E. coli* are the same as the mechanisms for the *recA*-dependent repair of DNA daughter-strand gaps during postreplication repair. The only significant difference is that the gaps arise in the DNA by different mechanisms during excision repair and postreplication repair.

This postulate would require that excision repair in unreplicated chromosomes (i.e., where no sister duplexes are present and no intrachromosomal recombination can occur) should be *recA*-independent. The data in Fig. 1 (open symbols) indicate that this is the case. Both the *dnaA* and *dnaA recA* strains repaired almost all of the excision gaps. The similarity between the results for the *dnaA* and *dnaA recA* strains cannot be due to the lack of induction of RecA protein in the *dnaA* strain at 42°C, since such induction has been shown to occur (Salles and Defais, 1984).

The average DNA content of *dnaA* cells that had been incubated for 3 h at 42.5°C to allow the completion of all rounds of DNA replication (Table 2) indicates that a substantial population of the cells contained 2 (or more) chromosomes. Using flow cytometry, Kogoma et al. (1985) have also found that a significant population of *dnaA* cells held at 42°C contain 2 chromosomes per cell. Since such cells show no *recA*-dependent excision repair (Fig. 1, open symbols), it suggests that recombination between chromosomes does not occur, at least in terms of the *recA*-dependent repair of excision gaps.

Our postulate also suggests that, if cells are UV irradiated when their chromosomes are partially replicated, excision repair should be partially *recA*

dependent, since intrachromosomal recombination can occur. This has also been shown to be the case (Fig. 1, closed symbols). Under these experimental conditions, the *dnaA recA* cells showed a large deficiency in the repair of excision gaps. Even the *dnaA* cells were somewhat deficient in the repair of excision gaps, suggesting that the *recA*-dependent repair of excision gaps is not as efficient as is the *recA*-independent repair of excision gaps. This result is consistent with the greater complexity of the *recA*-dependent versus the *recA*-independent process. In addition, the survival results (Fig. 3) show that the *dnaA* and *dnaA recA* cells were more UV-radiation sensitive when their chromosomes were partially replicated than when they were aligned (Fig. 3).

Even though the experiments reported here only used cells whose DNA was labeled prior to UV irradiation, it was important to determine if the movement of the replication fork and/or the process of postreplication repair affected our assay of excision repair in prelabeled DNA. We used a *dnaB*(Ts) strain to stop DNA synthesis after UV irradiation, and found that an active replication fork and the formation and repair of DNA daughter-strand gaps neither interfered with nor was required for the *recA*-dependent repair of excision gaps (Fig. 2). This conclusion is also supported by the data of Cooper (1982), who showed that repair replication after UV irradiation was the same for wild-type cells and for *dnaB*(Ts) cells at 42°C.

Studies on DNA-repair synthesis (Cooper and Hanawalt, 1972) have also indicated that there are two types of excision repair, and they can be distinguished by the sizes of the repair patches produced. The majority of the excisable lesions are repaired through short-patch repair that is *recA* independent. Only a small portion of the excisable lesions are repaired by long-patch repair. The long-patch repair process is inducible (Cooper, 1982), requires functional *recA* (Cooper and Hanawalt, 1972) and *recF* (Hanawalt et al., 1982) genes, but does not require a functional *recB* gene (Cooper, 1982). However, the repair of excision gaps in DNA is partially dependent on both the *recF* and *recB* genes, with *recF* playing the major role (Fig. 4). This result is similar to the relative importance of the *recF* and *recB* genes in postreplication repair, i.e., most of the gaps are repaired by a *recF*-dependent process, but a few gaps are converted to DNA double-strand breaks, which are then repaired by a *recB*-dependent process (Wang and Smith, 1983).

On the basis of these observations, we propose the following model for *recA*-dependent, growth-medium-dependent nucleotide-excision repair.

(i) When a lesion in the replicated portion of the chromosome is excised (Fig. 5C), the resulting gap can be repaired either by the *recA*-independent, growth-medium-independent, DNA polymerase-I-dependent process, or the excision gap may initiate recombination processes. In one such recombination process, a portion of the homologous strand in the adjacent sister duplex would be used to fill the excision gap (Fig. 5D, E) by the

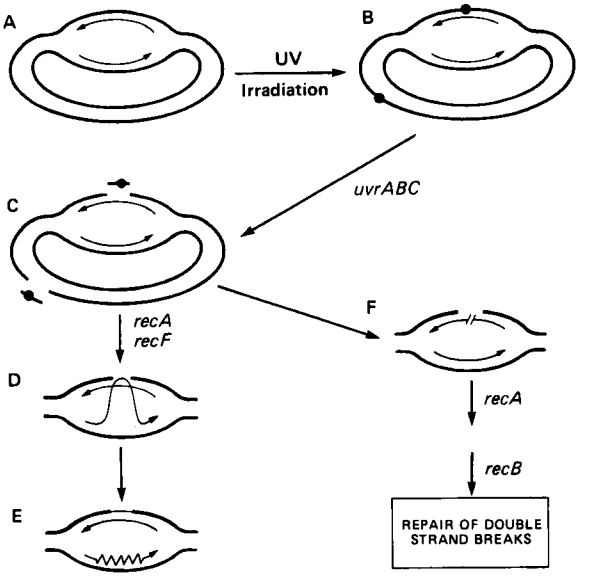

Fig. 5. Model for the *recA*-dependent repair of excision gaps in UV-irradiated *E. coli*. Lesions can be produced in both the replicated and nonreplicated regions of the chromosome (A, B), but only the excision gaps produced in the replicated region of the chromosome (C) should initiate an intrachromosomal recombinational process that is *recF* dependent (D). This process leaves a gap in the homologous sister duplex that can be filled by long-patch repair replication (wavy line), using the parental strand opposite the gap as a template (E). If the daughter strand opposite the excision gap is cut (—/—; F), the resulting double-strand break can be repaired by a *recB*-dependent process (see text for further discussion).

same *recF*-dependent process that functions to fill DNA daughter-strand gaps during postreplication repair (Ganesan and Seawell, 1975; Kato, 1977; Rothman and Clark, 1977; Wang and Smith, 1983). The resulting gap left in the sister duplex would be filled by long-patch repair replication (Fig. 5E). Thus, we suggest that the *recF*-dependent, long-patch excision-repair process is analogous to the *recF*-dependent repair of DNA daughter-strand gaps in postreplication repair; the only difference being in the manner in which the gaps are produced.

(ii) However, in the replicated portion of the chromosome, when the DNA strand opposite an excision gap is cut, we propose that the resulting double-strand break becomes susceptible to a *recB*-dependent repair process (Fig. 5F) that is analogous to the *recB*-dependent pathway for the postreplication repair of DNA double-strand breaks that arise at the sites of unrepaired DNA daughter-strand gaps (Wang and Smith, 1983, 1986).

Enzymatically induced DNA double-strand breaks accumulate in UV-irradiated excision-proficient cells (Bonura and Smith, 1975a, b), and are implicated as the major cause of lethality in UV-irradiated wild-type *E. coli* cells (Bonura and Smith, 1975b). These DNA double-strand breaks were presumed to be formed by overlapping excision gaps (Bonura and Smith, 1975a). However, in view of recent data suggesting that DNA double-strand breaks are produced in excision-deficient cells by cutting the parental strand opposite a DNA daughter-strand gap (Wang and Smith, 1986), such a process should also be considered to explain the double-strand breaks that arise as a consequence of excision repair. That is, a double-strand break can be produced at an excision gap by cutting the DNA strand opposite the gap.

In summary, the *recA*-dependent repair of excision gaps only occurs in the replicated portion of an *E. coli* chromosome. We find no evidence of interchromosomal recombination in the *recA*-dependent repair of excision gaps. The *recF*-dependent and *recB*-dependent pathways of excision repair are suggested to be analogous to the two major processes of postreplication repair, i.e., in the replicated portion of the chromosome, the RecF pathway repairs gaps, and the RecB pathway repairs the DNA double-strand breaks that arise at unrepaired gaps.

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