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Evidence that *recBC*-Dependent Degradation of Duplex DNA in *Escherichia coli recD* Mutants Involves DNA Unwinding

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Infection of *Escherichia coli* with phage T4 gene *2am* was used to transport ^{3}H-labeled linear duplex DNA into cells to follow its degradation in relation to the cellular genotype. In wild-type cells, 49% of the DNA was made acid soluble within 60 min; in *recB* or *recC* cells, only about 5% of the DNA was made acid soluble. Remarkably, in *recD* cells about 25% of the DNA was rendered acid soluble. The DNA degradation in *recD* cells depended on intact *recB* and *recC* genes. The degradation in *recD* cells was largely decreased by mutations in *recJ* (which eliminates the 5′ single-strand-specific exonuclease coded by this gene) or *xonA* (which abolishes the 3′ single-strand-specific exonuclease I). In a *recD recJ xonA* triple mutant, the degradation of linear duplex DNA was roughly at the level of a *recB* mutant. Results similar to those with the set of *recD* strains were also obtained with a *recC*‡ mutant (in which the RecD protein is intact but does not function) and its *recJ*, *xonA*, and *recJ xonA* derivatives. The observations provide evidence for a *recBC*-dependent DNA-unwinding activity that renders unwound DNA susceptible to exonucleolytic degradation. It is proposed that the DNA-unwinding activity causes the efficient recombination, DNA repair, and SOS induction (after application of nalidixic acid) in *recD* mutants. The RecBC helicase indirectly detected here may have a central function in Chi-dependent recombination and in the recombinational repair of double-strand breaks by the RecBCD pathway.

Apart from RecA protein, the RecBCD enzyme is the principal component of the major route of homologous recombination in *Escherichia coli* (5, 19, 37). In vitro, the enzyme has multiple enzymatic activities, including ATP-dependent exonuclease for double- and single-stranded DNA, ATP-stimulated endonuclease for single-stranded DNA, and ATP-dependent DNA-unwinding activity (40, 42). The *recB*, *recC*, and *recD* genes code for the three subunits of the RecBCD enzyme in *E. coli* (1, 2, 6) and other bacteria (22, 28, 50, 51). *recB* or *recC* null mutants of *E. coli* are recombination deficient and sensitive to UV light and other DNA-damaging agents (37) and are unable to derepress the SOS regulon after treatment of cells with nalidixic acid (9, 46). Unlike *recB* or *recC* null mutants, *recD* cells are UV resistant and recombination proficient but do not contain the highly active ATP-dependent duplex DNA exonuclease activity characteristic of cells with RecBCD enzyme (1, 2). It was proposed that a new pathway of recombination, RecY, is activated in *recD* mutants (19) and that this pathway depends on *recJ* function (13, 17). More important, *recD* mutants no longer respond to the hot spots of *recBCD*-dependent recombination called Chi (1, 3). A search for enzymatic activities of the RecBC core enzyme (without the RecD subunit) in cell extracts from strains that overproduce the RecBC enzyme did not reveal the nucleolytic and DNA-unwinding activities present in the RecBCD holoenzyme (36, 40). RecBC enzyme purified from cells with a Tn*1000* insertion in the *recD* gene had weak DNA helicase activity that was very sensitive to assay conditions (25). The RecBC enzyme reconstituted from over 90% purified RecB and RecC subunits had no detectable helicase or DNase activity on duplex DNA unless the purified RecD subunit was added (7). The ability of the RecBC enzyme to promote recombination and repair without displaying the activities characteristic of the RecBCD enzyme was an enigma (36, 40).

We studied the interaction of RecBCD and RecBC enzymes with linear duplex DNA in vivo. We used infection of cells with a phage T4 gene *2am* mutant (T4 2^-) to transport labeled linear duplex DNA into cells and followed its degradation. Phage T4 2^- lacks the gene *2* pilot protein on its DNA ends. This protein protects the phage genome from degradation by the RecBCD enzyme (24).

MATERIALS AND METHODS

Bacterial strains. The strains employed were mostly *E. coli* AB1157 and its derivatives (Table 1). The AB1157 genetic background was chosen because of its *supE44* suppressor of nonsense mutations. First, it suppresses the gene *2* amber mutation of T4 2^-, allowing undegraded genomes to be equipped with the gene *2* proteins necessary for subsequent successful infection of a *recB*$^+$ *C*$^+$ *D*$^+$ cell. Second, the suppressor does not suppress the nonsense allele *recD1011* (1) used in this study. The bacterial strains were constructed by P1 transduction. The selection for the *recJ284*::Tn*10* allele (15) was done on LB medium with tetracycline (15 μg/ml), and screening was done by testing the increased UV sensitivity in the *recD* genetic background (13, 17). The *recB21* (10), *recC1010*, and *recD1011* (1, 3) mutations were cotransduced (60 to 95%) with *argA*$^+$ or *argA81*::Tn*10*. The strains used for cotransductions with *argA*$^+$ had previously been transduced to *argE*$^+$. Transductants with *recC1010* or *recD1011* were identified by the increased survival of phage T4 2^- on them compared with its survival on AB1157. Transductants with *recB21* or *recC22* mutations were identified by their UV sensitivity and the increased survival of phage T4 2^-. The *xonA2* mutation was from strain JC8260 (26) and was cotransduced (about 70% cotransduction) with *his*$^+$. It was identified by crossing it into a derivative of JC8260 (like AB1157, but *recB21 recC22 xonA2 his*$^+$) in which the *xonA2* allele had been replaced by *xonA*$^+$ (strain

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TABLE 1. *E. coli* strains

Strain	Description[a]	Source or reference
WA234	AB1157: F⁻ *argE3 hisG4 leuB6 proA2*(Δ) *thr-1 thi-1 ara-14 galK2 lacY1 mtl-1 xyl-5 tsx-33 rpsL31 supE44*	10
BT125	As WA234, but *recD1011 arg*⁺	This work
WA632	As WA234, but *recB21 arg*⁺	This work
WA794	As WA234, but *recB21 recJ284*::Tn*10 arg*⁺	This work
BT122	As WA234, but *recJ284*::Tn*10*	This work
WA735	As BT234, but *recD1011 recJ284*::Tn*10 arg*⁺	This work
WA818	As WA234, but *xonA2 his*⁺ *arg*⁺	This work
WA821	As WA234, but *xonA2 recD1011 his*⁺ *argE*⁺ *argA81*::Tn*10*	This work
WA820	As WA234, but *xonA2 recJ284*::Tn*10 his*⁺ *arg*⁺	This work
WA822	As WA234, but *xonA2 recD1011 recJ284*::Tn*10 his*⁺ *arg*⁺	This work
WA819	As WA234, but *xonA2 recB21 his*⁺ *argE*⁺ *argA81*::Tn*10*	This work
WA800	As WA234, but *recC1010* (*recC*‡) *argE*⁺ *argA81*::Tn*10*	This work
WA805	As WA234, but *recC1010* (*recC*‡) *recJ284*::Tn*10 arg*⁺	This work
WA827	As WA234, but *recC1010* (*recC*‡) *xonA2 his*⁺ *argE*⁺ *argA81*::Tn*10*	This work
WA828	As WA234, but *recC1010* (*recC*‡) *xonA2 recJ284*::Tn*10 his*⁺ *arg*⁺	This work
WA440	KL187: F⁻ *thi-1 pyrD34 his-68 trp-45 mtl-2 xyl-7 malA1 galK35 strA118 recC22*	K. B. Low
WA676(pBR322)	Δ(*argA-recB*)*231 lacZ*::Tn*10 proA2*	50
WA676(pRR4)	Δ(*argA-recB*)*231 lacZ*::Tn*10 proA2*	This work
WA675(pBR322)	Δ(*argA-thyA*)*232 lacZ*::Tn*10 proA2*	50
WA675(pRR4)	Δ(*argA-thyA*)*232 lacZ*::Tn*10 proA2*	This work

[a] *recJ284*::Tn*10* was derived from strain JC12166 (14), *xonA2* was derived from strain JC8260 (22), *recD1011* was derived from strain V220 (1), *recC1010* (*recC*‡) was derived from strain V404 (3), and *recB21* was derived from strain AB2470 (10).

BT207; like JC8260, but *xonA*⁺ *his*::Tn*10*), rendering the strain UV sensitive. Reintroduction of *xonA2* restored the UV resistance to the level of resistance of JC8260.

Measurement of DNA degradation. Phage T4 *2*⁻ labeled with [³H]thymidine was prepared by standard procedures. The phage preparations used in this study had specific radioactivities of at least 10^3 cpm/10^8 infective centers and contained less than 1% acid-soluble radioactive material. The intracellular degradation of T4 *2*⁻ DNA was determined as previously described (45). The multiplicity of infection did not exceed 1. The amount of nonadsorbed phages was determined 10 min after adding phages to cells. At that time, the cells in the sample were sedimented and the total and acid-soluble radioactive material in the supernatant was quantified. The difference was considered the amount of nonadsorbed phages. Generally, it did not exceed 20% of the input radioactivity. The portion of nonadsorbed phages was taken into account when the intracellular degradation of DNA was calculated (45).

Determination of the EOP. The efficiency of plating (EOP) of T4 *2*⁻ was determined as follows. Phages (in 0.2 ml of LB) were allowed to adsorb to log-phase cells of the various strains (8×10^7 cells in 0.4 ml of LB plus 50 μg of tryptophan per ml) for 15 min at 30°C. The cells were sedimented and then resuspended in 0.6 ml of LB, and 0.3 ml was plated with soft agar. The nonadsorbed phages in the supernatant were quantified by plating with strain WA632. The EOP values in Table 3 were calculated by considering only the fraction of adsorbed phages.

RESULTS

DNA degradation in *recD* mutants. The kinetics and the final extent of degradation of T4 *2*⁻ DNA to acid-soluble material after phage infection of log-phase cells are shown in Fig. 1. In the wild type (AB1157), degradation started rapidly and resulted in 49% acid-soluble material after 60 min at 30°C. In AB1157 with the multicopy plasmid pDW1 carrying the *recBCD* genes of *E. coli*, resulting in about 20-fold overproduction of exonuclease V activity (31), the degradation was slightly faster and the final extent was 55% (data not shown). In *recB* mutant cells, DNA degradation occurred much more slowly and resulted in only 5.5% acid-soluble material (Fig. 1). In a *recC* mutant, 3.8% acid-soluble material was obtained (Table 2). This shows that in wild-type cells the breakdown of introduced linear duplex DNA is mainly caused by the RecBCD enzyme; this result is consistent with earlier observations (24, 35, 45). In a *recD* mutant, the initial rate and the extent of degradation after 60 min were about half of those obtained in wild-type cells (Fig. 1).

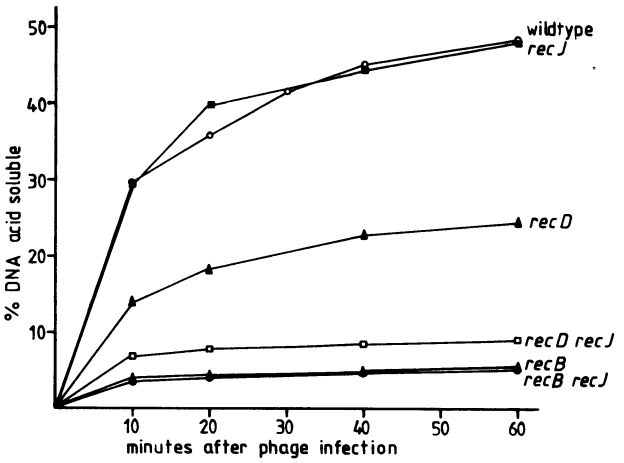

FIG. 1. Degradation of [³H]thymidine-labeled phage T4 *2*⁻ DNA in various *E. coli* strains after phage infection (multiplicity of infection, ≤1). After cells were mixed with phages, samples were removed at the times indicated and analyzed for acid-soluble and -insoluble material. The data are means of two or three experiments. They were corrected for incomplete phage adsorption as described in Materials and Methods. Symbols: ○, WA234 (wild type); △, WA632 (*recB*); ▲, BT125 (*recD*); ■, BT122 (*recJ*); □, WA735 (*recD recJ*); ●, WA794 (*recB recJ*).

TABLE 2. Degradation of [^{3}H]thymidine-labeled phage T4 2^- DNA in log-phase cells of various *E. coli* mutants

Strain	Relevant genotype	% of cold acid-soluble products[a]
WA440	*recC*	3.8
WA676(pBR322)	Δ*recBD*	3.5
WA675(pBR322)	Δ*recBCD*	4.7
WA676(pRR4)	Δ*recBD*/*recB*$^+$	32.2
WA675(pRR4)	Δ*recBCD*/*recB*$^+$	3.4
WA800	*recC*‡	14.5
WA805	*recC*‡ *recJ*	9.3
WA827	*recC*‡ *xonA*	9.4
WA828	*recC*‡ *recJ xonA*	7.2

[a] Determined 60 min after the addition of phage T4 2^- to the cells (for details, see the legend to Fig. 1 and Materials and Methods); the data are means of two or three determinations.

This was surprising, because according to published data, no significant exonuclease activity for duplex DNA would be expected in a *recD* mutant strain (1–3, 7, 25, 36, 40). Results similar to those with the *recD1011* point mutant strain were also obtained with a strain with a partial *recD* deletion (32% acid-soluble material after 60 min; Table 3). This strain [WA676(pRR4)] has a chromosomal deletion of the *recB* and *recD* genes (50) and contains a pBR328 plasmid with a 6.6-kb insert (consisting of a 4.1-kb *Cla*I-*Sal*I fragment and a 2.5-kb *Sal*I fragment) expressing RecB and an inactive truncated RecD protein that lacks 130 amino acids from the carboxy terminus (8, 30). This result argues against the possibility that the *recD1011* point mutation was leaky. The extensive DNA degradation in *recD* cells was abolished by the additional deletion of *recB* [strain WA676(pBR322)] or *recC* [strain WA675(pRR4)] or *recB* plus *recC* [strain WA675 (pBR322)] (Table 2). This indicated that the DNA degradation in *recD* strains was dependent on RecB and RecC proteins.

TABLE 3. EOP of phage T4 2^- on *E. coli* AB1157(WA234) (wild type) and various mutants[a]

Strain	Relevant genotype	Relative EOP of T4 2^-
WA632	*recB*	1
WA234	Wild type	0.0004
BT125	*recD*	0.042
BT122	*recJ*	0.0006
WA818	*xonA*	0.0006
WA820	*recJ xonA*	0.0006
WA735	*recD recJ*	0.15
WA821	*recD xonA*	0.022
WA822	*recD recJ xonA*	0.36
WA819	*recB xonA*	1
WA794	*recB recJ*	1
WA676(pBR322)	Δ*recBD*	0.81
WA676(pRR4)	Δ*recBD*/*recB*$^+$	0.01[b]
WA675(pBR322)	Δ*recBCD*	1
WA675(pRR4)	Δ*recBCD*/*recB*$^+$	0.84
WA440	*recC*	1
WA800	*recC*‡	0.061
WA805	*recC*‡ *recJ*	0.22
WA827	*recC*‡ *xonA*	0.022
WA828	*recC*‡ *recJ xonA*	0.50

[a] The EOP values are means of two to four determinations; in each determination, between about 100 and 400 plaques were counted on plates with the sedimented infected cells (see Materials and Methods for the procedure). The EOP of phage T4$^+$ on the strains varied by no more than 25%.

[b] Plaques of T4 2^- were minute, probably because the strain does not have a nonsense suppressor; plaques of T4$^+$ were normal.

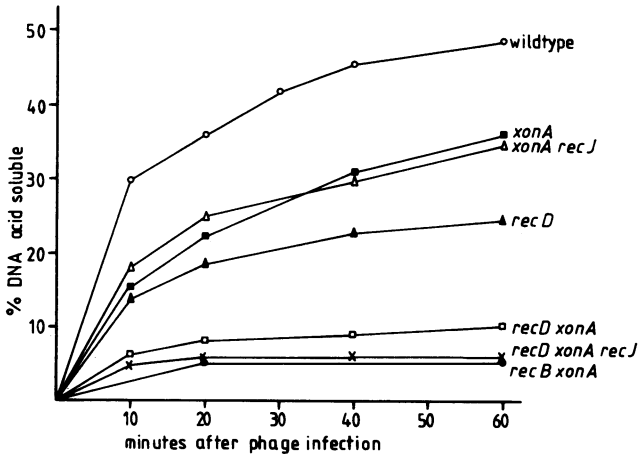

FIG. 2. Degradation of [^{3}H]thymidine-labeled phage T4 2^- DNA in various *E. coli* strains. For details, see the legend to Fig. 1. Symbols: ○, WA234 (wild type); ■, WA818 (*xonA*); △, WA820 (*xonA recJ*); ▲, BT125 (*recD*); □, WA821 (*recD xonA*); ×, WA822 (*recD recJ xonA*); ●, WA819 (*recB xonA*).

The survival of phage T4 2^- on *recB*, *recD*, and wild-type cells corresponded to the observed degrees of DNA degradation in these strains. The EOP (Table 3) on *recD* mutants [strains BT125 and WA676 (pRR4)] was intermediate (1×10^{-2} to 4×10^{-2}) relative to the full survival on the *recB* and *recC* mutants and the survival of 4×10^{-4} on wild-type cells. The deletion of *recB* [WA676(pBR322)], *recC* [WA675 (pRR4)], or both [WA675(pBR322)] in addition to a *recD* deletion always resulted an EOP of about 1 (Table 3), which is in accord with the low DNA degradation in these strains (Table 2).

Effects of *recJ* and *xonA* mutations. If the RecBC enzyme by itself does not degrade linear duplex DNA, it may process the DNA to render it available for other DNases. In a *recD recJ* double mutant, degradation was much lower (Fig. 1) and T4 2^- survival increased three- to fourfold relative to that of the *recD* strain (Table 3). The *recJ* gene codes for a single-strand-specific exonuclease with a preference for 5′ ends (16). In *recB*$^+$ *C*$^+$ *D*$^+$ *cells, a recJ* mutation did not decrease duplex DNA degradation (Fig. 1). A *xonA2* mutation known to completely eliminate exonuclease I (26) also caused a strong reduction of *recBC*-dependent DNA degradation (Fig. 2). Exonuclease I degrades single-stranded DNA from the 3′ end. Why the survival of T4 2^- was not correspondingly increased in a *recD xonA* double mutant (Table 3) is not understood. Similarly, an *sbcB15* mutation that greatly reduces exonuclease I activity (11) also decreased the degradation in a *recD* strain, although the effect was not as strong as that of the *xonA* mutation, and also did not improve the T4 2^- survival (data not shown). In agreement with previous observations (35), the lack of exonuclease I decreased the production of acid-soluble material in otherwise wild-type cells (Fig. 2). This is in accord with the fact that the RecBCD enzyme produces significant portions of single-stranded DNA fragments during degradation of duplex DNA (18). Overexpression of RecJ DNase from the multicopy plasmid pJC763 (15) did not further decrease the survival of T4 2^- in wild-type and *recD* strains (data not shown). Apparently, the normal amount of RecJ DNase suffices for the *recBC*-dependent destruction of phage ge-

nomes in cells infected by single phages. The combination of *recJ* and *xonA* mutations in a *recD* background decreased the DNA degradation to the low level of *recB* or *recC* mutants (Fig. 1 and 2). The EOP of T4 2^- on the triple mutant reached 36% of the EOP on a *recB* strain (Table 3). The combination of the *recB21* mutation (which eliminates *recB* and *recD* gene products because of a polar insertion in *recB* [1]) with *recJ* (strain WA794) or *xonA* (strain WA819) gave minimal levels of DNA degradation (Fig. 1 and 2) and full survival of T4 2^- (Table 3). This suggests that the residual DNA degradation seen in *recB* or *recC* mutants is not caused by exonuclease I or *recJ* DNase. Further, the results confirm the conclusion drawn above that the *recJ*- and *xonA*-dependent DNA degradation in *recD* mutants requires a functional RecBC enzyme.

The *recC*‡ phenotype. A class of *recC* mutations represented by *recC1010* is called *recC*‡ (37). The *recC*‡ mutants lack exonuclease V activity but are recombination proficient (1, 3). The *recC*‡ cells (mutant *recC1010*) degraded about 15% of infecting T4 2^- DNA (Table 2). This portion was significantly reduced after *recJ* or *xonA* mutations were introduced into the strain (Table 2). The portion was lowered almost to the level of *recB* or *recC* strains by the combination of *recJ* and *xonA* mutations (Table 2; Fig. 1). As with the *recD* mutants, the EOP of T4 2^- on the *recC*‡ strain was between that of the wild type and those of the *recB* and *recC* strains (Table 3). The survival of the phage was increased threefold by the presence of *recJ* and sevenfold by the presence of *recJ xonA* mutations (Table 3). Again, the combination of *recC*‡ with *xonA* did not improve the EOP of T4 2^- (Table 3).

DISCUSSION

The data presented herein show that the considerable degradation of linear duplex DNA in *recD* cells is *recBC* dependent and relies in addition on the action of single-strand-specific exonucleases, and in particular on the RecJ DNase (5′ specific) and exonuclease I (3′ specific). We conclude that, before single-strand hydrolysis, a *recBC*-dependent unwinding of duplex DNA must occur. It is likely that the RecBC core enzyme by itself has the necessary helicase activity in vivo. It is known that the RecBCD holoenzyme has helicase activity (32, 41), which becomes easily detectable under conditions (e.g., in the presence of Ca^{2+}) that inhibit the duplex DNA exonuclease activity (32). Although several in vitro studies of the RecBC core enzyme did not show an unwinding activity (7, 36, 40), one examination of a purified enzyme preparation identified weak helicase activity (25). On the other hand, it is also conceivable that in vivo some other, as yet unidentified protein could enable the RecBC core enzyme to perform DNA unwinding. The data in Fig. 1 and 2 and in Table 3 do not exclude the possibility that the RecJ protein or exonuclease I itself is that component. It is unclear why the degradation of duplex DNA observed here in vivo was not detected previously in extracts of *recD* mutants (36, 40). Perhaps the in vitro reaction conditions were inappropriate (e.g., the concentration of ATP was not adequate) or a functional association of RecBC enzyme with other proteins involved in the process was disrupted during extract preparation.

With respect to DNA degradation and phage T4 2^- survival, observations essentially similar to those with the *recD* mutant and its *recJ* and *xonA* derivatives were made with a *recC*‡ mutant (which is *recD*$^+$) and its derivatives. This supports the view that the intact RecC subunit effects the proper binding of the RecD protein to the RecBC core enzyme. Such a conclusion was drawn from recombination studies in *recC*‡ (36, 37) and from studies in which the titration of the RecD protein by excess RecC protein in vivo became apparent (29). This binding endows the enzyme with its duplex DNA exonuclease activity. The mutant RecC‡ protein, together with the intact RecB protein, apparently still supports to a significant extent the DNA helicase function typical of the RecBC core enzyme. In summary, it appears that in *recD* and *recC*‡ strains duplex DNA ends are attacked and unwound in a process that depends on both RecB and RecC proteins.

This interpretation of our results may help in understanding the recombination-proficient and UV-resistant phenotype of *recD* mutants. Smith (36, 37) considered that an as yet unidentified activity of the RecBCD enzyme was maintained in the RecBC enzyme and provided the cell with the functions for recombination and repair. Now it appears that the DNA unwinding indirectly detected here, coupled with the absence of exonuclease V activity, could be responsible for the high level of recombination in *recD* strains. Possibly, the unwinding activity of the RecBC core enzyme is identical to the helicase activity that is present in the complete RecBCD enzyme but is partially hidden by its nuclease function. The helicase activity could play a central role in the RecBCD pathway of recombination by producing recombinogenic single strands at the ends of duplex DNA. It was proposed previously that loss of the RecD subunit would convert the RecBCD enzyme into a powerful recombinase and that Chi sequences approached by the RecBCD enzyme in the correct orientation would increase the chance for a loss of RecD protein (38, 43). If the loss of the RecD protein in situ would uncover the helicase activity described here, then DNA unwinding could be the recombination-promoting function of the RecBCD enzyme or its modulated variant, the RecBC core enzyme. Duplex DNA unwinding by the RecBC core enzyme could constitute the principal process in a very convincing general model (split end model) for homologous recombination at double-strand ends and at Chi sequences recently put forward by Rosenberg and Hastings (33). The unwinding of duplex ends would facilitate the recombinational repair of double-strand breaks by use of an intact homologous chromosome (33, 39) into which the single strands could invade in a RecA protein-dependent process (27). DNA unwinding by RecBC core enzyme would start directly at the DNA end. Unwinding by the complete RecBCD enzyme would start after trimming the duplex to the next Chi sequence, which causes the loss of the RecD subunit. Such a mechanism could also explain the wild-type-like UV resistance of *recD* mutants. Single-strand gaps opposite unexcised pyrimidine dimers are known to be converted frequently into double-strand breaks (49), which can subsequently be repaired with the aid of the duplex unwinding activity of the RecBCD or RecBC enzyme according to the split end model (33, 39).

If the 5′ single strand exposed during unwinding of a duplex end is degraded by the RecJ DNase, then the resulting 3′ strand protruding from the duplex would match with the polarity of the RecA protein-catalyzed strand exchange (27). This could explain the facts that *recJ* mutations depress conjugational recombination in *recD* mutants and block repair of UV-damaged DNA dependent on RecBC enzyme and RecA protein (13, 17, 47). However, hyperrecombination of λ DNA in *recD* mutants is *recJ* independent (44). Whether mutations eliminating exonuclease I affect recombination in *recD* mutants is not known.

The production of single strands by *recBC*-dependent unwinding would also explain the efficient induction of the SOS regulon in *recD* mutants by nalidixic acid, an agent that is suspected to promote double-strand breaks and that does not induce the SOS response in *recB* and *recC* mutants (4). Single-stranded DNA is assumed to be an SOS-inducing signal, because RecA protein binds to it and thereby is activated into a LexA protein-cleaving coprotease (12). The possibility of a *recBC*-dependent DNA-unwinding process was considered when the nalidixic acid-induced SOS response of *recD* mutants was detected (4).

Finally, it is known that double mutants of *recB* (or *recC*) with *polA* or *dam* are not viable (20, 23). We propose that the reason for this is the inefficient repair of double-strand breaks in strains deficient for *recB*, *recC*, or both (34). Such breaks probably accumulate in *polA* strains because of cleavages within persisting single-strand gaps and in *dam* strains because of unbiased nicking of parental and newly synthesized DNA strands in the course of mismatch repair. The observation that *recD polA* and *recD dam* strains are viable (48) and that *recD* cells are even more resistant than wild-type cells to gamma irradiation (21) are in accord with the proposal that *recBC*-dependent unwinding of duplex DNA ends initiates efficient recombination repair at double-strand breaks.

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