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Genetics

DNA double-strand break repair: Genetic determinants of flanking crossing-over

(gene conversion/homologous recombination/*ruvC*/*recJ*)

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ABSTRACT **Whether or not homologous interaction of two DNA molecules results in crossing-over of the flanking sequences is an important decision in view of genome organization. Several homologous recombination models, including the double-strand break repair models, explain this decision as choice between two alternative modes of resolution of Holliday-type intermediates. We have demonstrated that a double-strand gap can be repaired through gene conversion copying a homologous duplex, as predicted by the double-strand break repair models, in the RecE pathway of *Escherichia coli*. This gap repair is often accompanied by crossing-over of the flanking sequences. Mutations in *ruvC* and *recG*, whose products interact with Holliday structures *in vitro*, do not block double-strand gap repair or its association with flanking crossing-over. However, two mutations in the *recJ* gene, which encodes a single-strand 5′ → 3′ exonuclease, severely decrease association of flanking crossing-over. Two mutations in the *recQ* gene, which encodes a helicase, moderately decrease association of flanking crossing-over by themselves and suppress the severe effect of a *recJ* mutation. Similar relationships of *recJ* and *recQ* mutations are observed in cell survival after ultraviolet light irradiation, γ-ray irradiation, and H_2O_2 treatment. We discuss how cooperation of the *recQ* gene product and the *recJ* gene product brings about double-strand break repair accompanied by flanking crossing-over. We also discuss how this reaction is related to repair of chromosome damages.**

Homologous interaction between two DNA segments may or may not result in crossing-over of the flanking sequences (Fig. 1*A*). Since such crossing-over could cause gross changes in genome organization, such as deletion, inversion, and translocation, the choice between crossing-over and non-crossing-over may be an important decision. The Holliday model and its descendant models of homologous recombination explain this decision as a choice between two alternative modes of resolution of Holliday structure or related intermediate structures (1–3). In fact, some Holliday resolvases recognize specific sequences at the Holliday joint and promote only one mode of resolution (4). But it is not known whether this reflects such a choice *in vivo*.

The double-strand break repair models (Fig. 1*A*) (2, 5) propose that homologous recombination is initiated by a double-stranded break on one of the two DNA duplexes. The double-stranded break is repaired by copying a homologous duplex. In the models the intermediates are resolved with or without crossing-over of the flanking sequences (Fig. 1*A*). We have demonstrated in *Escherichia coli* the double-strand break repair reaction predicted by these models (5–8).

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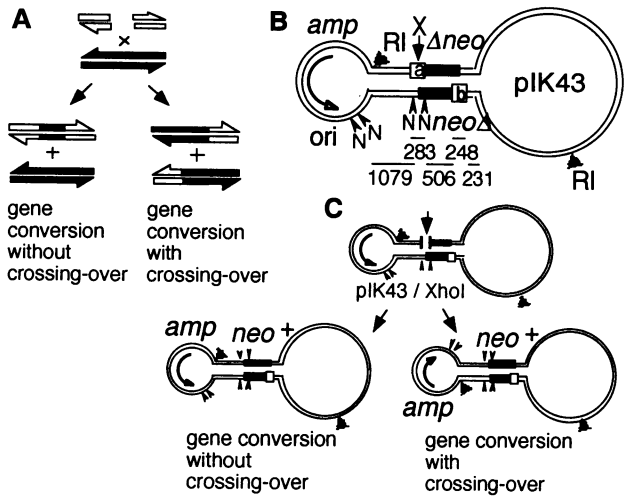

FIG. 1. (*A*) Double-strand gap repair by gene conversion. A double-strand gap in duplex DNA (upper) is repaired by copying homologous DNA (center). Two types of product pairs are produced. One pair is gene conversion with crossing-over of the flanking sequences (right). The other pair is gene conversion without crossing-over of the flanking sequences (left). (*B*) Substrate plasmid, pIK43. The two homologous duplex segments are drawn as parallel lines. The *neo* gene (stippled box) is from Tn*5*. The top segment has a deletion (deletion a) between the two *Nae* I sites, which removed one end (the C end) of the *neo* gene. The *Nae* I site was inactivated by insertion of an *Xho* I linker sequence, 5′-CCTCGAGG. The bottom segment has a deletion (deletion b), which removed the other end. The left unique part (2321 bp) is derived from pBR322. The entire length is 14795 bp. The restriction enzymes and their site coordinates are as follows: X, *Xho* I, 1082; N, *Nae* I, 11109, 11392, 12590, 12750; RI, *Eco*RI, 8024, 14793. (*C*) Double-strand gap repair experiment. Cutting pIK43 with *Xho* I (arrow) produces a double-stranded gap of ≈283 bp (compared with the lower segment) in the upper segment. The double-stranded gap is repaired by copying the homologous sequence of the lower segment within the transferred cells. This repair event restores a *neo*$^+$ gene and makes the host cell resistant to kanamycin (Kan^R). The gap repair results in loss of the *Xho* I site and in generation of two *Nae* I sites. The repair may take place with (right) or without (left) crossing-over of the flanking sequences. Crossing-over inverts the left unique part and alters the *Eco*RI pattern.

In this work we show that this repair is frequently accompanied by crossing-over. We looked for genetic determinants affecting the crossing-over association with particular interest in two proteins, RuvC and RecG, recently shown to catalyze processing of Holliday structure *in vitro* (9–11). We

Abbreviations: Kan^R, kanamycin-resistant; Amp^R, ampicillin-resistant.

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found, however, mutations in RecG and/or RuvC proteins do not affect efficiency of the double-strand break repair reaction or its association with crossing-over. Instead, we found that *recJ* and *recQ* functions (12, 13) promote associated crossing-over. We also obtained evidence for functional interaction of RecJ and RecQ proteins *in vivo*. A similar relationship was observed in repair of chromosomal damages by several agents.

MATERIALS AND METHODS

Double-Strand Gap Repair by Transformation. The methods were detailed previously (5, 8). The parental plasmid, pIK43, was purified by banding in cesium chloride/ethidium bromide. Plasmids were cut thoroughly with *Xho* I. The cut and uncut plasmids were used to transform various *E. coli* strains by the rubidium chloride method as described (8) except that the cells were grown in SOB medium (14). Immediately after the transformation reaction, including incubation for 60 min, aliquots of the transformation mixture were spread directly on ampicillin agar plates or on kanamycin agar plates. Numbers of the transformant colonies per transformation reaction were plotted. Plasmids were then recovered from these transformants by an alkaline method (15) and were analyzed with restriction enzymes (16).

UV Sensitivity Measurement. Exponential-phase cultures in L broth were diluted in M9 salts (17) and spread on L agar plates. The plates were irradiated with UV light (254 nm) for various times. Colonies were scored after incubation at 37°C for 24 hr in the dark.

γ-Ray Sensitivity Measurement. Exponential-phase cultures in L broth were diluted in M9 salts and spread on L agar plates. The plates were irradiated with γ-rays [cobalt, 105 rad/min (1 rad = 0.01 Gy)] for various times. Colonies were scored after incubation at 37°C for 24 hr.

H_2O_2 Sensitivity Measurement. Exponential-phase cultures in L broth were harvested and resuspended in M9 salts at 2 × 10^8 cells per ml. Various amounts of H_2O_2 were added and the cells were incubated for 20 min at 37°C. The cells were diluted in M9 salts without H_2O_2 to terminate the reaction and then spread on L agar plates. Colonies were scored after incubation at 37°C for 24 hr.

RESULTS

Frequent Association of Flanking Crossing-Over with Double-Strand Gap Repair. Fig. 1*A* illustrates the double-strand gap repair by gene conversion that we examined (5). Our substrate plasmid (pIK43) carries, in inverted orientation, two copies of a sequence containing the *neo* gene (Fig. 1*B*). The lower segment has a deletion removing one end of *neo*. The upper segment has a deletion that removes the other end of *neo*. Because an oligonucleotide containing a site for restriction enzyme *Xho* I is inserted at the latter deletion, cleavage of this plasmid with *Xho* I generates a long double-stranded gap corresponding to the deletion (≈300 bp long) when compared with the lower sequence. (There is some terminal heterology, derived from the linker nucleotide between the cut sequence and the "lower" sequence of the *neo* gene. These are 5′-CC and 3′-GGAGCT at the left end and 5′-TCGAGG and 3′-CC at the right end.) We introduce this gapped plasmid into the *E. coli* cells and immediately spread the cells on agar plates containing kanamycin. If conservative recombination reaction repairs this gap, using the corresponding wild-type sequence in the lower segment as a template (Fig. 1*A*), the two types of products in Fig. 1*C* will be produced. These products carry a functional neo^+ gene and thus confer the host cell resistance to kanamycin. Overall transformation efficiency was also measured by counting ampicillin-resistant (Amp^R) transformant colonies as our substrate plasmid carries the *amp* gene.

Our results in the first row of Fig. 2 confirm and extend our earlier results with a *recBC sbcA* strain (5). The ratio of the Kan^R transformants to the Amp^R transformants was 10^{-4} to 10^{-3} with intact substrate plasmid (pIK43). Double-strand gap formation with *Xho* I increased the yield of Kan^R transformants 30- to 100-fold. Plasmid molecules were recovered from each of these Kan^R transformants from the cut plasmid. Restriction enzyme analysis (with *Xho* I, *Nae* I, and *Eco*RI; see Fig. 1*B*) showed that all of the transformants examined carried plasmids predicted by the double-strand break repair models (Fig. 1*C*) (5). The numbers of the Amp^R transformants also indicated occurrence of the double-strand gap repair reaction. The *Xho* I cut decreased Amp^R transformants only 10- to 30-fold (Fig. 2, row 1). The level of these Amp^R transformants was close to that of the Kan^R transformants. Indeed, most of the Amp^R transformants carried the gene-conversion-type products (Fig. 1*C*) (5). The *sbcA* mutation activates the RecE pathway of homologous recombination by derepressing *recE* and other genes (23). A *recE159* derivative of the *recBC sbcA* strain is defective in the double-strand gap repair reaction (6, 7). With this strain (Fig. 2, row 2), (*i*) cutting the input plasmid decreased Amp^R transformants severely (≈1000-fold) and (*ii*) the Kan^R recombinants remained at a low level even after the cut. These properties are like those of a nonisogenic *recA* strain (5).

The repair took place with (Fig. 1*C*, right) or without (Fig. 1*C*, left) crossing-over of the flanking sequences. The cross-

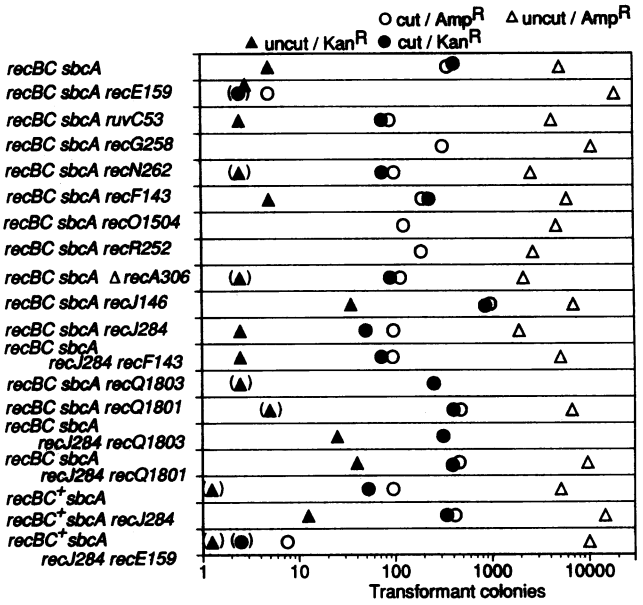

FIG. 2. Double-strand gap repair in various *E. coli* strains carrying Rac prophage. The strains were transformed with 500 ng of uncut pIK43 or *Xho* I-cut pIK43 by a rubidium method (5, 8). Immediately after the transformation reaction, including incubation for 60 min, aliquots of the transformation mixture were spread on ampicillin agar plates or on kanamycin agar plates. Numbers of transformant colonies per reaction were plotted. One set of results from two or three independent experiments is shown for each strain. The first strain is JC8679 (6, 18), which is *recB21 recC22 rac*$^+$ (Rac prophage present) *sbcA23* and F$^-$ *hisG4 thr-1 leuB6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 argE3 rfbD1 mgl-51 kdgK51* Δ*(gpt-proA)62 rpsL31 tsx-33 supE44* λ$^-$. The others are its derivatives described earlier (6) or gifts [the *recQ1801* derivative from S. Lovett (19) and the *recJ146* derivative from A. J. Clark (20)]. The remainder were by P1 transduction with the following donors: KD2245 [*ruvC53 eda51*::Tn*10*, from H. Nakayama (21)], JC12123 [*recJ284*::Tn*10*, from A. J. Clark (20)], NK5992 [*argA81*::Tn*10*, from A. F. Taylor (22)].

ing-over can be detected with *Eco*RI digestion (Fig. 1*C*). We analyzed >100 Kan^R clones and found that 71% of the gap repair events were accompanied by flanking crossing-over (Fig. 3, top row).

Mutations in Holliday Interacting Proteins, RuvC and RecG, Do Not Block Double-Strand Gap Repair or Associated Crossing-Over. We next looked for mutations that block this double-strand gap repair or affect its association with crossing-over. We were particularly interested in two genes, *ruvC* and *recG*, since RuvC protein resolves Holliday structure *in vitro* (9, 10) and since RecG protein dissociates Holliday structure *in vitro* (11). A *ruvC53* derivative of the *recBC sbcA* strain showed a decrease in recombination proficiency in conjugation (24) and increased sensitivity to UV (K.K. and I.K., unpublished results). This strain showed capacity for double-strand gap repair as judged by two criteria (Fig. 2, row 3): (*i*) cutting of the substrate plasmid increased the Kan^R recombinants about 50-fold and (*ii*) cutting decreased Amp^R transformants only about 50-fold. These patterns resemble those of the *recBC sbcA* strain rather than that of the *recE* derivative. Analysis of the product plasmids with restriction enzymes showed that association of flanking crossing-over was as frequent as in the *recBC sbcA* strain (Fig. 3*A*, row 2). A *recG* derivative of the *recBC sbcA* strain showed a decrease in recombination proficiency in conjugation and increased sensitivity to UV (25). A *recG258*::*kan* derivative of the *recBC sbcA* strain showed a capacity for double-strand gap repair similar to that in the *ruvC* derivative. Cutting of the substrate plasmid decreased Amp^R transformants only moderately (Fig. 2, row 4). All Amp^R transformants (45/45) carried the gene-conversion-type products (Fig. 1*C*). Association of flanking crossing-over was as frequent as in the *recBC sbcA* strain (Fig. 3*A*, row 3).

Mutations in *recJ* Exonuclease Decrease Association of Flanking Crossing-Over. We found that this double-strand gap repair capacity of the *recBC sbcA* strain was not abolished by the following mutations judged by the above criteria: *recN262*, *recF143*, *recO1504* (insertion), *recR252* (insertion), *recA306* (deletion), *recQ1803* (insertion, formerly *recQ107*), *recQ1801*, *recJ146*, *recJ284* (insertion) (Fig. 2 and ref. 6). In the *recN262*, *recF143*, *recO1504*, *recR252*, and *recA306* derivatives, association of crossing-over with repair was as frequent as in the parental (*recBC sbcA*) strain (Fig. 3*A*). In the *recJ146* derivative and the *recJ284* derivative, however, association of crossing-over with repair was much decreased (Fig. 4*B*). The *recJ* product is a 5′ → 3′, single-strand-specific exonuclease (12). The *recJ146* mutation confers a thermosensitive defect in conjugational recombination and repair of UV light damage (20). But the decrease of the crossing-over was seen even at 32°C, a permissive temperature (6/43 repair products with crossing-over), as well as at 42°C, a nonpermissive temperature (3/47 repair products with crossing-over).

The RecF pathway promotes apparent gene conversion without crossing-over by a different mechanism (26, 27). We asked whether the products in the above *recJ* derivatives were made by the RecE pathway or by another mechanism—the RecF pathway, for example. A *recF143* version of the *recBC sbcA recJ284* strain turned out to be proficient in the double-strand gap repair (Fig. 2). Association of the crossing-over was as rare as in the *recBC sbcA recJ284* strain (Fig. 3*B*). Thus, the RecF pathway is not responsible for the gap repair in this strain. We then asked about *recE* dependence of the gap repair in the *recJ* derivative. A *recE159* version of the *recBC sbcA recJ284* strain turned out to be poor in transformation. Therefore, we examined the *recBC*$^+$ versions. A *recBC*$^+$ *sbcA23* strain showed the double-strand gap repair capacity (Fig. 2). This repair was frequently accompanied by crossing-over (29/54). The *recJ284* mutation did not abolish this double-strand gap repair ability (Fig. 2) but decreased the accompanying crossing-over (4/45). A *recE159* derivative of this strain was deficient in the gap repair (Fig. 2, last row). We

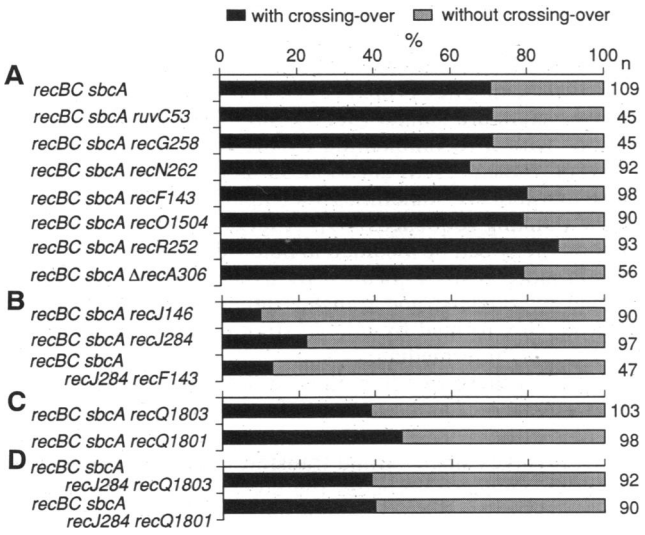

FIG. 3. Effect of various mutations on association of flanking crossing-over with the double-strand gap repair. (*A*) Mutations with no detectable effect. (*B*) *recJ* derivatives. (*C*) *recQ* derivatives. (*D*) *recJ recQ* derivatives. The repaired plasmid molecules were prepared from each of the Kan^R transformants (or the Amp^R transformants for strains already Kan^R) obtained by transformation with pIK43 cut with *Xho* I. They were classified with *Eco*RI as shown in Fig. 1*C*. *n*, Number of the total colonies analyzed. (A colony giving both of the two types contributed 0.5 unit to both types. These colonies did not exceed 5% of the total colonies in any of the strains.)

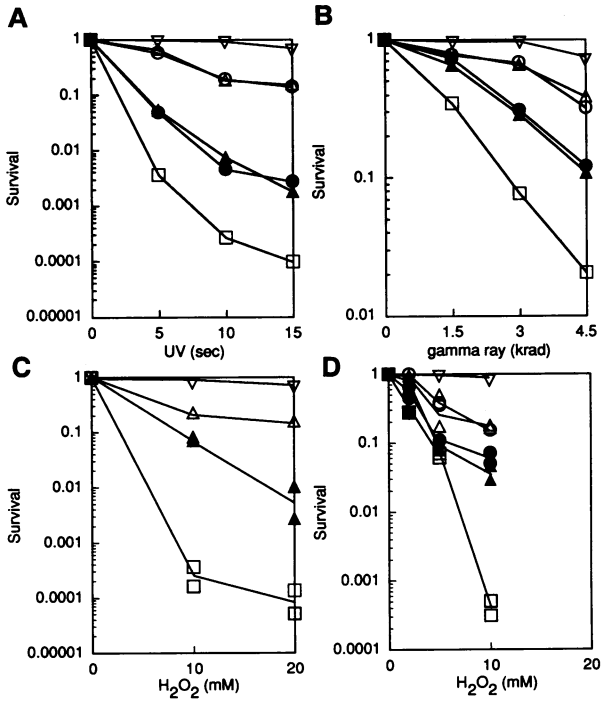

FIG. 4. Suppression of repair deficiency in a *recBC sbcA recJ* strain by *recQ* mutations. (*A*) UV. (*B*) γ-Ray. (*C* and *D*) H_2O_2. Measurements were carried out for two independent clones from each of the strains. Average values from two clones are plotted in *A* and *B*. *C* and *D* were carried out on different days. ▽, *recBC sbcA*; ○, *recBC sbcA recQ1801*; △, *recBC sbcA recQ1803*; □, *recBC sbcA recJ284*; ●, *recBC sbcA recJ284 recQ1801*; ▲, *recBC sbcA recJ284 recQ1803*.

concluded that the gap repair altered by the *recJ* mutation still proceeds via the RecE pathway.

Mutations in RecQ Helicase Moderately Decrease Association of Flanking Crossing-Over and Suppress the Severe Defect with a *recJ* Mutation. A *recQ* mutation, in a gene coding for a helicase (13), decreases recombination in conjugation and increases UV sensitivity in the *recBC sbcA* background (19). We found that two mutations in the *recQ* gene, *recQ1801* and *recQ1803*, also decreased crossing-over association (Fig. 3*C*). The decrease was not as severe as that observed with the *recJ* mutations. But this does not appear to be the result of leakiness of the mutations because *recQ1801* shows a typical null phenotype and because *recQ1803* is a product of Tn*3* insertion into the coding region (28). Two strains carrying the *recJ284* mutation and one of these *recQ* mutations showed a level of crossing-over association characteristic of the *recQ* mutants and not of the *recJ* mutant (Fig. 3*D*). In other words, these *recQ* mutations suppressed the severe defect of the *recJ* mutation. These results suggested the possibility that RecJ exonuclease and RecQ helicase play roles at nearby steps in the double-strand gap repair reaction associated with flanking crossing-over and led us to the following experiments.

Similar Suppression of the *recJ* Mutation by the *recQ* Mutations in Repair of Damage by UV Light, γ-Ray, and H_2O_2. Qualitatively similar relationships between the *recJ* mutation and the *recQ* mutations were observed in survival after damage by UV, γ-ray, and H_2O_2. These include (*i*) severe defect with a *recJ* mutation, (*ii*) only slight defect with the *recQ* mutations, and (*iii*) suppression of the *recJ* defect by the *recQ* mutations (Fig. 4).

The *recJ* mutations make the cells sensitive to UV in the *recBC sbcA* background (ref. 20; Fig. 4*A*). The *recQ* mutants were only slightly sensitive in this background (ref. 20; Fig. 4*A*). The two *recQ* mutations suppressed this defect of the *recJ* derivative in the *recBC sbcA* background (Fig. 4*A*). A mutation in another RecF pathway gene, the *recF143* mutation, did not show such suppression and had only a modest additive effect on the *recJ* mutation (data not shown). γ-Ray irradiation introduces double-stranded breaks on *E. coli* chromosomes (29). The *recJ284* mutation made the host cell sensitive in the *recBC sbcA* background (Fig. 4*B*). The two *recQ* mutations conferred slight sensitivity by themselves and suppressed the above sensitivity (Fig. 4*B*). The level of suppression was again partial. Such suppression was not observed with the *recF143* mutation (data not shown). The *recJ284* mutation made the host cell sensitive to H_2O_2 in the *recBC sbcA* background (Fig. 4 *C* and *D*). The two *recQ* mutations suppressed this sensitivity (Fig. 4*C*). The effects of H_2O_2 depended on its concentration. The above relationship was reproducibly observed at 10 mM (Fig. 4 *C* and *D*). Such suppression was not observed with the *recF143* mutation (data not shown). These results suggest that RecJ and RecQ cooperate in repair of DNA damages and that RecQ operates before RecJ.

DISCUSSION

We showed that RecJ function and RecQ function are determinants of association of flanking crossing-over with the conservative double-strand gap repair by homologous recombination. The following provides clues to their modes of action.

(*i*) Decrease of association of crossing-over was strong with the *recJ* mutations but not so strong with the *recQ* mutations (Fig. 3). In the *recJ recQ* double derivatives, the decrease was as in the *recQ* derivatives (Fig. 3). In other words, the *recQ* mutations partially suppressed the effect of the *recJ* mutation. This suppression was gene-specific and not allele-specific with respect to the *recQ* mutation (Fig. 3). These suggest the following. There are two routes to double-strand break repair with flanking crossing-over. One major route (defined here as route A) requires RecQ and RecJ functions. RecQ operates before RecJ. The other bypass route (defined as route B) does not require them. Inactivation of RecQ opens the bypass route B. There should be a third route (route C) leading to double-strand break repair *without* flanking crossing-over. Route C is independent of RecJ and RecQ functions.

(*ii*) The *recE* mutation blocks double-strand break repair completely whether or not it is associated with flanking crossing-over (Fig. 2; ref. 6). RecE exonuclease (exonuclease VIII) and RecT protein (30) are both essential (7). Two multicopy plasmids carrying *recE* and *recT* genes show enhanced capacity of the double-strand gap repair reaction (7). We assume that RecE exonuclease and RecT protein may play essential, early, and rate-limiting roles. They might be digestion from the ends to expose the 3′ single strand and annealing of it with a homologous duplex (5, 31).

(*iii*) Alternative resolution of Holliday-type intermediates has been envisioned as underlying choice of association of flanking crossing-over (2, 3). Holliday-type structures were detected in *E. coli* (32, 33). RuvC protein resolves Holliday structures *in vitro* (9, 10), and RecG protein promotes branch migration *in vitro* (11). But mutations in RuvC or RecG did not block the double-strand break repair or its association with flanking crossing-over (Figs. 2 and 3). A *recBC sbcA ruvC recG* strain was also proficient in the double-strand gap repair and associated crossing-over (K.K. and I.K., unpublished observation). Although there might be another enzyme that is responsible for resolution of Holliday-type intermediates, with and without flanking crossing-over, in the double-strand break repair reaction (34), we might suppose an alternative view to the choice of association of flanking crossing-over.

(*iv*) λ's own Red pathway of homologous recombination resembles the RecE pathway (35). In fact, analysis of λ *cos* led to the proposal of a double-strand break repair model (5, 8, 18, 31). Two λ-coded proteins, λ exonuclease and *redβ* annealing protein, are sufficient to confer double-strand gap repair capacity to *E. coli* (8). Likewise, two proteins coded by Rac prophage, RecE exonuclease and RecT annealing protein, are sufficient to confer double-strand gap repair capacity to *E. coli* lacking Rac prophage (7). The activities of these two enzyme pairs are similar (30, 36–38). Therefore, the observations with λ recombination by the Red pathway may be relevant to the present question. In fact, λ recombinants for close markers are sometimes accompanied by crossing-over of the flanking sequences (39). A large (≈700 bp) region of nonhomology can be included in heteroduplex products (40). This is more easily explained by asymmetric heteroduplex formed by single-strand transfer rather than by symmetric heteroduplex formed by double-strand branch migration of the Holliday intermediates (1).

Based on these, we hypothesize the following to explain the effects of *recJ* and *recQ* mutations. Route C, which leads to repair without crossing-over independently from RecJ and RecQ, involves single-strand transfer. (*i*) RecE exonuclease and other nucleases process the ends at the double-strand break. (*ii*) RecT anneals the ends with one single strand from a homolog. (*iii*) Repair synthesis follows. This route may not involve Holliday structure and hence does not require RuvC or RecG.

We hypothesize that route A, the RecJQ-dependent route, involves symmetric heteroduplex formation and proceeds as follows. (*i*) RecE exonuclease processes the ends at the break. (*ii*) RecT pairs the resulting single strand with 3′ end with a homologous duplex. (*iii*) RecQ helicase, or another helicase, further opens the cut duplex, uncut duplex, or both duplexes and initiates strand transfer. (*iv*) RecJ exonuclease

digests the 5′ end single strand and thus accelerates this strand transfer and double-stranded branch migration. (*v*) Repair synthesis follows. (*vi*) The resulting Holliday-like structure can be resolved with flanking crossing-over (or, possibly, without flanking crossing-over). A Holliday resolving enzyme other than RuvC (34) might be involved in this step. Formation of double-strand break might favor this route and results in higher frequency of crossing-over than that observed in λ recombination (39).

We further hypothesize that the absence of RecQ function leads to activation of route B, by allowing action of other helicases and exonucleases at the ends. Recently, deletion of *helD*, coding for a helicase, or of *uvrD*, coding for another helicase, was shown to lead to a defect in recombination in conjugation in *recBC sbcA* background (41). We cannot exclude the other possibilities for RecJ and RecQ action. For example, RecJ exonuclease might be directly involved in processing of the intermediate form.

How are these findings in a plasmid model system related to the chromosomal repair? Qualitatively similar relationships between the *recJ* mutation and the *recQ* mutations were observed in cell survival after treatment with three DNA damaging agents (Fig. 4). These include (*i*) severe defect with a *recJ* mutation, (*ii*) only slight defect with the *recQ* mutations, and (*iii*) suppression of the *recJ* defect by the *recQ* mutations. This parallelism suggests that the roles of RecJ function and RecQ function and their relationships in these DNA repair processes might be similar to those in the double-strand break repair reaction with flanking crossing-over.

In *E. coli* RecF pathway, apparent gene conversion is not accompanied by crossing-over (26). This apparent conversion is explained by successive rounds of nonconservative recombination (27) or single-strand transfer. Similar mechanisms may make possible chromosomal gene conversion without flanking crossing-over (3). In mitotic recombination in the yeast *Saccharomyces*, longer gene conversion tracts are preferentially associated with flanking crossing-over (42). Mutations in a gene for helicase cause hyper-gene-conversion and suppress UV sensitivity of mutations in another gene in *Saccharomyces* (43, 44).

Why costly processes of homologous recombination and sex have evolved has been a mystery. It has been proposed that homologous recombination has evolved because it is beneficial for repair (45). This hypothesis can easily explain gene conversion (without flanking crossing-over). But it has not been clear why flanking crossing-over is necessary for repair. Our results demonstrate that there are gene products promoting flanking crossing-over and that these products are also required for repair. This suggests the essential nature of the link between repair and crossing-over.

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