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Roles of RecJ, RecO, and RecR in RecET-Mediated Illegitimate Recombination in *Escherichia coli*

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We analyzed effects of overexpression of RecE and RecT on illegitimate recombination during prophage induction in *Escherichia coli* and found that frequencies of spontaneous and UV-induced illegitimate recombination are enhanced by coexpression of RecE and RecT in the wild type, but the enhanced recombination was reduced by *recJ, recO*, or *recR* mutation. The results indicated that RecET-mediated illegitimate recombination depends on the functions of RecJ, RecO, and RecR, suggesting that the RecE and RecJ exonucleases play different roles in this recombination pathway and that the RecO and RecR proteins also play important roles in the recombination. On the other hand, the frequency of the RecET-mediated illegitimate recombination was enhanced by a *recQ* mutation, implying that the RecQ protein plays a role in suppression of RecET-mediated illegitimate recombination. It was also found that RecET-mediated illegitimate recombination is independent of the RecA function with UV irradiation, but it is enhanced by the *recA* mutation without UV irradiation. Based on these results, we propose a model for the roles of RecJOR on RecET-mediated illegitimate recombination.

Illegitimate recombination is a major cause of chromosomal aberration, along with duplication, deletion, insertion, and translocation. Illegitimate recombination spontaneously occurs at a low frequency, but it is greatly enhanced by treatment with UV light or other DNA-damaging agents (10). This observation indicates that DNA lesions introduced by DNA-damaging agents cause illegitimate recombination.

Illegitimate recombination is a class of recombination that takes place between sequences of little or no homology, and it results in DNA rearrangements. Illegitimate recombination can be classified into two classes, short-homology-independent illegitimate recombination (SHIIR) and short-homology-dependent illegitimate recombination (SHDIR) (20, 21, 23, 27). SHIIR occurs between sequences with virtually no homology and is mediated by DNA topoisomerases (1, 20). SHDIR is induced by UV irradiation or other DNA-damaging agents and requires short regions of homology between recombination sites. These regions usually contain 4 to 10 bp of homologous DNA (23, 27). It has been shown that RecJ exonuclease promotes SHIIR, but RecQ helicase suppresses it (9, 23). RecE is also known to play a role in illegitimate recombination (13, 26).

In DNA double-strand break repair mediated by the RecE pathway, RecE and RecT play a central role in the recombinational repair, and many other Rec proteins, including RecA, RecF, RecJ, RecO, RecR, RecQ, and RuvC, are involved in it (2, 4, 6, 15). The *recE* gene encodes a 5′-to-3′ double-stranded-DNA-specific exonuclease which selectively degrades the 5′ end of DNA, producing a duplex with a 3′ overhang (11, 12, 14). RecT promotes annealing of complementary single DNA strands and can catalyze the formation of joint molecules (7, 8). The *recJ* gene encodes an exonuclease which is characterized as a 5′-to-3′ single-stranded-DNA-specific exonuclease (17). It has been suggested that RecJ processes the double-stranded ends of DNA with RecQ helicase to produce 3′ single-stranded DNA ends, as does RecE. RecFOR promotes annealing of complementary single-stranded DNA and strand exchange in RecA-mediated homologous recombination (18, 24, 25).

In this study, we showed that overexpression of RecE and RecT enhances the frequencies of spontaneous and UV-induced illegitimate recombination and that the functions of RecJ, RecO, and RecR are required for this RecET-mediated illegitimate recombination. In addition, we also found that the function of RecQ plays a role in suppression of RecET-mediated illegitimate recombination. A model for RecET-mediated illegitimate recombination is discussed.

MATERIALS AND METHODS

Bacterial strains. The *Escherichia coli* strains used in this study are described in Table 1. All strains in this work are derivatives of *E. coli* K-12, which contains one unit of the λ *c*I857 prophage except for Ymel and P2 lysogen. Ymel was used for titration of the total number of λ phage, and P2 lysogen was used for titration of the number of λ Spi$^-$ phage. Plasmid pRAC3 is a pBR322-based plasmid containing *E. coli recE*$^+$*T*$^+$ genes (19), and plasmids pJC1501 and pJC1509 are pBR322-based plasmids containing the *E. coli recE*$^+$gene (4). These plasmids were kindly provided by I. Kobayashi. Plasmid pRDK579 is a pET28a$^+$ plasmid containing the *E. coli recE*$^+$ gene, and plasmid pRDK577 is a pET24$^+$ plasmid containing the *E. coli recT*$^+$ gene (R. D. Kolodner, personal communication). These plasmids were kindly provided by R. D. Kolodner.

Media and conditions of growth of bacteria and phage. λ YP broth contained 10 g of Bacto tryptone (Difco), 1 g of yeast extract, 2.5 g of NaCl, 1.5 g of Na_2HPO_4, and 0.18 g of $MgSO_4$ in 1 liter of water and was used to grow bacteria and to detect λ*bio* transducing phage. λ trypticase agar contained 10 g of Trypticase Peptone (Becton Dickinson), 5 g of NaCl, and 12 g of agar in 1 liter and was used to titrate Spi$^-$ phage. λ agar contained 10 g of Bacto tryptone (Difco), 2.5 g of NaCl, and 12 g of agar in 1 liter and was used to titrate total λ phage.

Measurement of frequency of λ Spi$^-$ phage induced spontaneously or by UV irradiation. *E. coli* λ *c*I857 or its derivatives were grown at 30°C in λ YP broth. If necessary, 2 ml of the culture was irradiated with a UV lamp (15 W) with a

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TABLE 1. *E. coli* strains used in this study

Strain	Description	Source or construction
Ymel	*supE supF*	Our collection
WL95	*supE supF metB trpR hsdR tonA* (P2)	Our collection
HI2898[e]	*sup*^{0} (λ *c*I857)/pBR322	HI1162
HI2899[a]	*sup*^{0} (λ *c*I857)/pRAC3 (*recE*$^+$ *T*$^+$)	HI1162
HI3023[c]	*sup*^{0} (λ *c*I857)/pRDK579 (*recE*$^+$)	HI1162
HI3024[d]	*sup*^{0} (λ *c*I857)/pRDK577 (*recT*$^+$)	HI1162
HI3033[b]	*sup*^{0} (λ *c*I857)/pJC1509 (*recE*$^+$)	HI1162
HI3034[b]	*sup*^{0} (λ *c*I857)/pJC1501 (*recE*$^+$)	HI1162
HI2900	*sup*^{0} *recJ284*::*tet* (λ *c*I857)/pBR322	HI2898 × P1(HI1346)
HI2901	*sup*^{0} *recJ284*::*tet* (λ *c*I857)/pRAC3 (*recE*$^+$*T*$^+$)	HI2899 × P1(HI1346)
HI3036	*sup*^{0} *recA306*::*tet* (λ *c*I857)/pBR322	HI2435
HI3035	*sup*^{0} *recA306*::*tet* (λ *c*I857)/pRAC3 (*recE*$^+$*T*$^+$)	HI2435
HI3038	*sup*^{0} *recQ101*::*cat* (λ *c*I857)/pBR322	HI2898 × P1(N1126 Δ*recQ101*::*cat*)
HI3037	*sup*^{0} *recQ101*::*cat* (λ *c*I857)/pRAC3 (*recE*$^+$*T*$^+$)	HI2899 × P1(N1126 Δ*recQ101*::*cat*)
HI3046	*sup*^{0} *recR252*::Tn/*O*minikan (λ *c*I857)/pBR322	HI2139
HI3047	*sup*^{0} *recR252*::Tn/*O*minikan (λ *c*I857)/pRAC3 (*recE*$^+$*T*$^+$)	HI2139
HI3062	*sup*^{0} *recO1504*::Tn*5* (λ *c*I857)/pBR322	HI3059
HI3063	*sup*^{0} *recO1504*::Tn*5* (λ *c*I857)/pRAC3 (*recE*$^+$*T*$^+$)	HI3059

[a] Plasmid pRAC3 is a pBR322 plasmid containing *E. coli recE*$^+$*T*$^+$ genes (22).
[b] Plasmid pJC1501 and pJC1509 are pBR322 plasmids containing *E. coli recE*$^+$ (4).
[c] Plasmid pRDK579 is a pET28a$^+$ plasmid containing *E. coli recE*$^+$ (R. D. Kolodner, personal communication).
[d] Plasmid pRDK577 is a pET24$^+$ plasmid containing *E. coli recT*$^+$ (R. D. Kolodner, personal communication).
[e] All λ lysogens contain one unit of λ prophage.

wavelength of 254 nm. If a strain carrying pRDK577 or pRDK579 was used, isopropyl-β-D-thiogalactopyranoside (0.2 mM; Sigma) was added to the culture 30 min before thermal induction. Thermal induction of λ prophage was carried out by incubation at 42°C for 15 min. The culture was then incubated at 37°C for 2 h. The titer of λ Spi$^-$ phage was measured on a lawn of *E. coli* WL95 (P2). The number of total λ phage was measured on a lawn of *E. coli* Ymel. The frequency of illegitimate recombination was calculated by dividing the number of λ Spi$^-$ phage by the total number of λ phage (10).

Determination of localization and nucleotide sequence of the recombination junctions in λ Spi$^-$ phage *E. coli* λ lysogen was irradiated with UV as described above. To isolate a single clone of λ Spi$^-$ phage, the culture was then divided into 50 small tubes. Each tube containing 0.5 ml of the culture was then incubated at 42°C for 15 min. The culture was then incubated at 37°C for 2 h. Phage lysates were plated on a lawn of *E. coli* WL95. A plaque derived from each tube was picked up, suspended in M9 buffer, and plated on a lawn of Ymel to isolate a single clone.

λ*bio* transducing phage was identified by PCR with a mixture of two sets of primers. Locations of recombination junctions were also determined through PCR by using multiple combinations of primers (23). The PCR products were directly sequenced using an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer).

RESULTS

Coexpression of RecE and RecT enhances frequencies of spontaneous and UV-induced illegitimate recombination. Frequencies of illegitimate recombination during induction of *E. coli* λ *c*I857 lysogens were measured using the λ Spi$^-$ phage assay (10). Specialized transducing phages generated from the λ prophage by illegitimate recombination usually comprise the *E. coli* genes *gal* or *bio*, which are adjacent to the phage genome. Most of the transducing phages are defective in the *red-gam* region of their genome, which enables them to form plaques on an *E. coli* P2 lysogen lawn (Spi$^-$ phenotype), in contrast to normal λ phage which cannot. Thus, it is possible to select λ Spi$^-$ phage from the phage pool. The number of λ Spi$^-$ phage after induction of a lysogen is assumed to be the same as that of λ*bio* transducing phage, since previous experiments have shown that most λ Spi$^-$ phages are λ*bio* phages (10, 27). Thus, determining the frequency of λ Spi$^-$ phage after induction of a lysogen estimates the frequency of illegitimate recombination.

We first examined the effect of expression of the *recET* genes on illegitimate recombination during prophage induction. Since the *E. coli* K-12 594 strain which was used as the wild type in this study does not contain the *recE* and *recT* genes, we introduced the pRAC3 plasmid carrying the *recE* and *recT* genes into the wild-type strain. It is known that pRAC3 contains the C-terminal domain of the *recE* gene and the *recT* gene (19), but it maintains the functions that promote homologous and illegitimate recombination (22, 26). Using these strains, we measured the frequency of λ Spi$^-$ phage after prophage induction and found that it is enhanced by the introduction of pRAC3 with or without UV irradiation (Table 2). On the other hand, the introduction of pRDK579 or pRDK577 that contains only the *recE* gene or the *recT* gene, respectively, did not increase the frequency of λ Spi$^-$ phage with or without UV irradiation (Table 2). The introduction of pJC1501 or pJC1509, which contain only the *recE* gene, also did not increase the frequency of λ Spi$^-$ phage (data not shown). These results indicate that coexpression of RecE and RecT is required for the enhancement of frequencies of spontaneous and UV-induced illegitimate recombination.

Roles of RecJ, RecO, RecR, and RecQ in RecET-mediated illegitimate recombination. Next, to understand the role of RecJ in RecET-mediated illegitimate recombination, we measured the frequency of λ Spi$^-$ phage in the *recJ*-deficient strain carrying pRAC3. Consistent with previous results (23), the results indicated that the frequency of λ Spi$^-$ phage was reduced in the *recJ*-deficient strain with pRAC3 compared to those of the *recJ*-proficient strain under UV-irradiated and unirradiated conditions (Table 2). This result indicates that the illegitimate recombination enhanced by RecE and RecT depends on the function of RecJ, with or without UV irradiation. Furthermore, consistent with the previous results (10), the

TABLE 2. Effects of overexpression of RecET and defect of ReeJ on frequency of λ Spi$^-$ phage

UV dose (J/m^2)	Strain[a]	Relevant mutation	Plasmid	Spi$^-$ phage (10^7)/ total λ phage[c] (SE)[d]	Rate relative to control	Burst size[b] (SE)
0	HI2898	Wild type	pBR322	0.013 (0.007)	1	156 (19)
0	HI2899	Wild type	pRAC3 (*recE*$^+$*T*$^+$)	0.092 (0.010)	7.1	105 (18)
0	HI3023	Wild type	pRDK579 (*recE*$^+$)	0.010 (0.001)	0.75	147 (17)
0	HI3024	Wild type	pRDK577 (*recT*$^+$)	0.013 (0.003)	1	150 (14)
0	HI2900	*recJ284*	pBR322	<0.01 (ND)[d]	<1	125 (25)
0	HI2901	*recJ284*	pRAC3 (*recE*$^+$*T*$^+$)	<0.01 (ND)[d]	<1	85 (25)
50	HI2898	Wild type	pBR322	0.92 (0.04)	1	98 (23)
50	HI2899	Wild type	pRAC3 (*recE*$^+$*T*$^+$)	11 (7)	12	50 (17)
50	HI3023	Wild type	pRDK579 (*recE*$^+$)	1.9 (0.1)	2.1	56 (14)
50	HI3024	Wild type	pRDK577 (*recT*$^+$)	1.8 (0.3)	2.0	47 (17)
50	HI2900	*recJ284*	pBR322	0.19 (0.05)	0.20	77 (15)
50	HI2901	*recJ284*	pRAC3 (*recE*$^+$*T*$^+$)	0.53 (0.24)	0.58	53 (18)
100	HI2898	Wild type	pBR322	1.8 (0.3)	1	30 (7.2)
100	HI2899	Wild type	pRAC3 (*recE*$^+$*T*$^+$)	23 (3)	13	28 (4.7)
100	HI3023	Wild type	pRDK579 (*recE*$^+$)	2.4 (0.2)	1.3	33 (3.6)
100	HI3024	Wild type	pRDK577 (*recT*$^+$)	2.5 (0.2)	1.4	29 (5.6)
100	HI2900	*recJ284*	pBR322	0.45 (0.17)	0.25	38 (12)
100	HI2901	*recJ284*	pRAC3 (*recE*$^+$*T*$^+$)	1.3 (0.4)	0.72	28 (8.5)

[a] All strains are *E. coli* K-12 594 or its derivatives containing one unit of λ *c*I857 prophage.
[b] Burst size indicates the number of total phage per cell.
[c] All numbers are averages of four determinations.
[d] ND, not determined: SE, standard error of the mean.

frequency of RecET-mediated illegitimate recombination was not affected by the *recA* mutation under UV irradiation, indicating that DNA-damage-induced illegitimate recombination enhanced by RecET is independent of the RecA function. Unexpectedly, the *recA* mutation enhanced the frequency of RecET-mediated illegitimate recombination without UV irradiation, implying that the defect of double-strand break repair mediated by the RecA function induces spontaneous illegitimate recombination which is enhanced by RecET (Table 3).

Because it is known that RecO, RecR, and RecQ are also involved in homologous recombination in the RecE pathway (15), we examined the effects of the *recO, recR,* and *recQ* mutations on RecET-mediated illegitimate recombination. We measured the frequency of λ Spi$^-$ phage in the *recO* mutants or *recR* mutants with or without pRAC3 and found that the frequency of λ Spi$^-$ phage was reduced in the *recO- or recR*-deficient strains with pRAC3 compared to those in the wild-type strain, with or without UV irradiation (Table 3). There was no effect on the control strains with the pBR322 plasmid, regardless of UV irradiation. This result indicates that RecET-mediated illegitimate recombination requires the functions of RecO and RecR, with or without UV irradiation.

Next, we measured the frequency of λ Spi$^-$ phage in the *recQ* mutant with or without UV irradiation. Consistent with previous results (9), we found that RecET-mediated illegitimate recombination is suppressed by the function of RecQ regardless of UV irradiation (Table 3).

Nucleotide sequences of recombination junctions produced in the RecET-mediated illegitimate recombination Next, we determined the distribution of recombination junctions of λ Spi$^-$ phage isolated from the RecET-expressing strain under UV irradiation. Since illegitimate recombination takes place between *E. coli bio* genes and λ *git-gam* regions as shown in Fig. 1A, the recombination junctions were amplified by PCR with several primer oligonucleotide sets, followed by agarose gel electrophoresis analysis. In the phages derived from the RecET-expressing *recJ*$^+$ strain, relative rates of recombination at hotspots I and III were 46 and 34%, respectively (Table 4). On the other hand, in the phages derived from the RecET-expressing *recJ* mutant, relative rates of recombination at hotspots I and III were 6% and less than 2%, respectively (Table 4). These results indicated that the RecJ function is required for RecET-mediated recombination at hotspots I and III under UV-irradiated conditions.

The junctions of the λ*bio* transducing phages EU15 and JEU21 resulted from recombination at Hotspot I, which is the same hotspot found previously by Yamaguchi et al. (27). At this hotspot, the recombination sites are known to share a short region of homology of 9 bp, as shown in Fig. 1B (panel a). The hotspot sites II and III share a short region of homology of 13 and 5 bp, respectively, as shown in Fig. 1B (panels b and c). Figure 1B (panels d to f) shows the junction sequences of λ*cbio* transducing phages derived from recombination at non-hotspot sites in the RecET-expressing *recJ*$^+$ strain. All recombination sites shared short regions of homology between *E. coli* and λ phage DNA (average length of homology, 7.6 bp). Therefore, the results confirmed previous results which suggest that RecET-mediated illegitimate recombination is short-homology dependent (13, 26). Figure 1B (panels g to k) shows the junction sequences of λ*bio* transducing phage derived from recombination at non-hotspot sites in the RecET-expressing *recJ* mutant. One group of the non-hotspot sites shared a short region of homology (average length of homology, 8.3 bp), but another group shared no or two homologous bases (average length of homology, 1.3 bp) (Fig. 1B, panels g to i). Therefore, in the RecET-expressing *recJ* mutant, illegitimate recombination could be at least partly mediated by a mechanism different

TABLE 3. Effect of the *recA, recO, recR*, and *recQ* mutations on spontaneous or UV-induced RecET-mediated illegitimate recombination

UV dose (J/m^2)	Strain[a]	Relevant mutation	Plasmid	Sp^- phage (10^7) per total λ phage[c] (SE)[d]	Rate relative to control	Burst size[b] (SE)
0	HI2898	Wild type	pBR322	0.015 (0.002)	1	140 (27)
0	HI3036	*recA306*	pBR322	0.010 (ND)	0.67	82 (11)
0	HI3038	*recQ101*	pBR322	0.16 (0.03)	11	100 (23)
0	HI3062	*recO1504*	pBR322	0.010 (ND)	0.67	150 (18)
0	HI3046	*recR252*	pBR322	0.012 (0.003)	0.80	120 (17)
0	HI2899	Wild type	pRAC3 ($recE^+T^+$)	0.14 (0.02)	1	130 (15)
0	HI3035	*recA306*	pRAC3 ($recE^+T^+$)	2.5 (0.8)	18	67 (13)
0	HI3037	*recQ101*	pRAC3 ($recE^+T^+$)	2.2 (0.04)	16	82 (13)
0	HI3063	*recO1504*	pRAC3 ($recE^+T^+$)	0.028 (0.006)	0.20	130 (20)
0	HI3047	*recR252*	pRAC3 ($recE^+T^+$)	0.013 (0.006)	0.09	120 (14)
50	HI2898	Wild type	pBR322	1.4 (0.2)	1	90 (20)
50	HI3036	*recA306*	pBR322	0.77 (0.15)	0.55	38 (7)
50	HI3038	*recQ101*	pBR322	9.9 (0.3)	7.0	44 (18)
50	HI3062	*recO1504*	pBR322	1.4 (0.3)	0.98	78 (14)
50	HI3046	*recR252*	pBR322	1.3 (0.3)	0.94	62 (24)
50	HI2899	Wild type	pRAC3 ($recE^+T^+$)	18 (1)	1	57 (17)
50	HI3035	*recA306*	pRAC3 ($recE^+T^+$)	11 (1)	0.62	40 (5)
50	HI3037	*recQ101*	pRAC3 ($recE^+T^+$)	92 (7)	5.0	37 (11)
50	HI3063	*recO1504*	pRAC3 ($recE^+T^+$)	2.4 (0.4)	0.13	59 (5)
50	HI3047	*recR252*	pRAC3 ($recE^+T^+$)	0.77 (0.09)	0.04	58 (19)
100	HI2898	Wild type	pBR322	1.9 (0.2)	1	35 (8)
100	HI3036	*recA306*	pBR322	1.3 (0.2)	0.71	26 (4)
100	HI3038	*recQ101*	pBR322	11 (2)	5.9	29 (6)
100	HI3062	*recO1504*	pBR322	2.7 (0.8)	1.4	33 (5)
100	HI3046	*recR252*	pBR322	1.7 (0.5)	0.92	28 (5)
100	HI2899	wild type	pRAC3 ($recE^+T^+$)	25 (2)	1	28 (8)
100	HI3035	*recA306*	pRAC3 ($recE^+T^+$)	14 (1)	0.57	20 (2)
100	HI3037	*recQ101*	pRAC3 ($recE^+T^+$)	160 (23)	6.5	28 (8)
100	HI3063	*recO1504*	pRAC3 ($recE^+T^+$)	3.2 (0.5)	0.13	25 (3)
100	HI3047	*recR252*	pRAC3 ($recE^+T^+$)	2.1 (0.6)	0.08	26 (8)

[a] All strains are *E. coli* K-12 594 or its derivatives containing one unit of λ *c*I857 prophage.
[b] Burst size indicates the number of total phages per cell.
[c] All numbers are averages of four determinations.
[d] ND, not determined; SE, standard error of the mean.

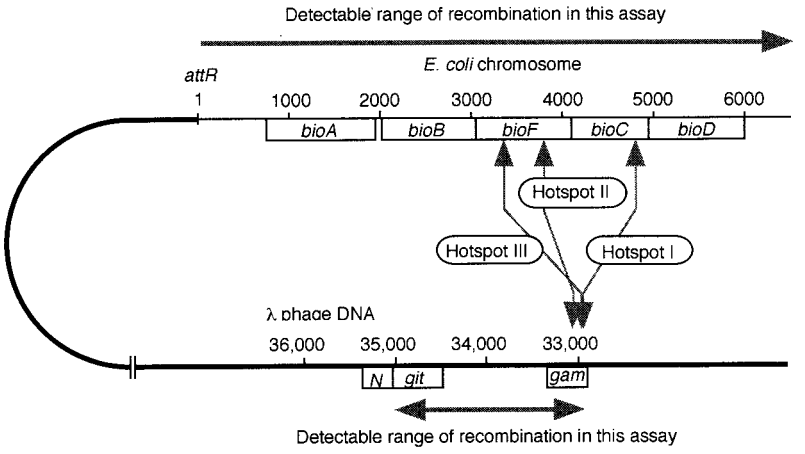
from that in the RecET-expressing $recJ^+$ strain. This point should be clarified in future studies.

DISCUSSION

We found that overexpression of RecE and RecT promotes spontaneous and UV-induced illegitimate recombination in the λ Spi^- assay system. Yamaguchi et al. (26) have previously shown that the RecE function is necessary for illegitimate recombination in their plasmid assay system. In addition to RecE, we also showed that coexpression of RecT is required for the enhancement of illegitimate recombination by RecE. RecE is a 5′-to-3′ double-stranded-DNA-specific exonuclease (11, 12, 14) and is able to restore homologous recombination in the *recBC* mutation background (1). RecT has an activity that promotes annealing of complementary single-stranded DNAs and possibly competes with single-stranded DNA binding protein (SSB) for binding to the RecE-generated 3′-single-strand overhangs, protecting them from digestion by ExoI (8). In fact, overexpression of ExoI suppresses the RecE-mediated illegitimate recombination (26). It is therefore suggested that RecE may digest 5′ ends of blunt-ended DNA and may produce a

FIG. 1. Nucleotide sequences of the recombination junctions of λ*bio* transducing phages isolated from the RecET-expressing strain. (A) Schematic representation of the recombination sites in this assay. (B, row i, panel a) Sequences of hotspot I detected at the recombination junctions of λ*bio* transducing phages EU15 and JEU21, which were isolated from the RecET-expressing wild type (HI2899) and *recJ* mutant (HI2901), respectively. The sequences shown in bold indicate homology at the recombination sites. Map coordinates for phage and bacterial sequences are indicated. (Row i, panel b) Sequences of hotspot II detected at the recombination junctions of λ*bio* transducing phages EU1 and JEU7, which were isolated from the RecET-expressing wild type and *recJ* mutant, respectively. (Row i, panel c) Sequences of hotspot III detected in the recombination junctions of λ*bio* transducing phage EU16, which was isolated from the RecET-expressing wild-type cells. (Row ii, panels d to f) Sequences of non-hotspot sites detected in the recombination junctions of λ*bio* transducing phages derived from the RecET-expressing wild type (HI2899). (Row iii, panels g to k) Sequences of non-hotspot sites derived from the RecET-expressing *recJ* mutant (HI2901).

A



B

(i) Hotspots

(a) Hotspot I

EU15, JEU21

```
        4771—bio—4785
        |           |
bio     GCTCAGTGCCATGCGTTCGCTGAAAGGCAT
        ||||||||||||||  ::   ::   :
λbio    GCTCAGTGCCATGCGCTCCTGAAACTCAAC
        :    ||||||||||||||||||||||||
λ       GTGTTCTGCCATGCGCTCCTGAAACTCAAC
             |                      |
          32882———λ———32905
```

(b) Hotspot II

EU1, JEU7

```
        3725———bio———3746
        |            |
bio     AACCAGAATTGCTGGTAGTGACTTTTGGCA
        ||||||||||||||||||||||||||
λbio    AACCAGAATTGCTGGTAGTGACGCGCCCAG
             :  ||||||||||||||||||||||||
λ       CGGGCGAGCTGCTGGTAGTGACGCGCCCAG
             |                      |
          33051——λ——33076
```

(c) Hotspot III

EU16

```
        3316———bio———3337
        |            |
bio     GAAGAAGAGCTGGCCGAGTGGCTTGGCTAT
        ||||||||||||||||||||||| : :: :
λbio    GAAGAAGAGCTGGCCGAGTGGCGTTGCAAA
        ::      :::::::  ||||||||||||||
λ       GATTTTTTGCTGGCCCCGTGGCGTTGCAAA
                         |           |
                      32948—λ—32960
```

(ii) Non-hotspots from *recE*+ strain

(d) Non-hotspot

EU16, EU22, EU29

```
        3454———bio———3472
        |            |
bio     TTGCTGGAAGCTGCCAGTTTAAGCCCGTCG
        ||||||||||||||||||  : : :
λbio    TTGCTGGAAGCTGCCAGTTCTGCCTCTTTC
        :   :  ||||||||||||||||||||||||
λ       TCCATGTCGTCTGCCAGTTCTGCCTCTTTC
                |                    |
             33016——λ——33035
```

(e) Non-hotspot

EU9

```
        3344———bio——3361
        |            |
bio     TATTCGCGGGCACTGCTGTTTATCTCTGGT
        ||||||||||||||||||  :  :  :  :
λbio    TATTCGCGGGCACTGCTGGTAGTGACGCGC
        :  :: :  ||||||||||||||||||||||
λ       TTCACGGGCGAGCTGCTGGTAGTGACGCGC
                |                    |
             33050——λ——33067
```

(f) Non-hotspot

EU4

```
        4572———bio———4590
        |            |
bio     ATCGGGTGGTGCGCCCCAAAGGCGTGGTCG
        ||||||||||||||||  :  ::
λbio    ATCGGGTGGTGCGCCCCGTCGAGCTGTATC
        : :  ||||||||||||||||||||||||
λ       GATTTTTTGCTGGCCCCGTCGAGCTGTATC
                |                    |
             32943——λ——33960
```

(iii) Non-hotspots from *recJ*- *recE*+ strain

(g) Non-hotspot

JEU2

```
        3815———bio———3830
        |            |
bio     TTACGTGCGTCGCTGGCGGTCATTCGCAGT
        ||||||||||||||||
λbio    TTACGTGCGTCGCTGGGCAAAGAAGATTTC
           :  :  :  ||||||||||||||||
λ       GCCCTCACACTGGAGGGCAAAGAAGATTTC
                      |              |
                   33948——λ——33963
```

(h) Non-hotspot

JEU15

```
        3362—bio—3377
        |        |
bio     TCTCTGGTTTCGCCGCTAATCAGGCAGTTA
        ||||||||||||||||  :  :
λbio    TCTCTGGTTTCGCCGCCACTATCAGGCAGC
        :  :     :  ||||||||||||||||||
λ       TGCCAAAAATAAAGGCCACTATCAGGCAGC
                   |                 |
                34078—λ—34093
```

(i) Non-hotspot

JEU33

```
        3362—bio—3376
        |        |
bio     TAAGCCATCATCCGCAAATTATCCGTGCCT
        ||||||||||||||  : :::: :
λbio    TAAGCCATCATCCGCCCACTATCAGGCAGC
        ::      ::     ||||||||||||||||
λ       TGCCAAAAATAAAGGCCACTATCAGGCAGC
                       |              |
                    34081— λ—34096
```

(j) Non-hotspot

JEU13, JEU19

```
        3633——bio——3651
        |           |
bio     TTCACGGGCGAGCTGCTGGTAGTGACGCGC
        ||||||||||||||||||  ::
λbio    TTCACGGGCGAGCTGCTGGCTGCAAAAGGT
           :: :  ||||||||||||||||||||||
λ       GGGGCGCGGCAGCTGCTGGCTGCAAAAGGT
                |                    |
             33048——λ——33067
```

(k) Non-hotspot

JEU47

```
        3094——bio——3152
        |           |
bio     GCCGGACGCTGGCTGGTGGCGGATGATCGC
        ||||||||||||||||||  :  :  :
λbio    GCCGGACGCTGGCTGGTGGTGAGCAATGGT
           :  :  ||||||||||||||||||||
λ       ATCAATATCAACCTGGTGGTGAGCAATGGT
                |                    |
             32840——λ—32857
```

TABLE 4. Distribution of recombination sites of λ*bio* transducing phages formed following UV irradiation[a]

Strain	Relevant mutation	Plasmid	No. of phage tested	Rate (%) of recombination at:			
				HotI	HotII	HotIII	Non-hot
HI2898	Wild type	pBR322	48	33	21	2	44
HI2899	Wild type	pRAC3 (*recE*$^+$ *T*$^+$)	50	46	4	34	16
HI2900	*recJ284*	pBR322	49	6	2	<2	92
HI2901	*recJ284*	pRAC3 (*recE*$^+$ *T*$^+$)	48	6	10	<2	83

[a] The distributions of recombination sites of recombinant phages derived from various strains were determined by PCR. The junctions were classified into four classes: hotspots I (Hot I), II (Hot II), and III (Hot III), and non-hotspot (Non-hot). The dose of UV irradiation was 100 J/m^2.

duplex DNA with a 3′ single-strand overhang, which may anneal the other end with the 3′-single-strand overhang in the presence of RecT.

Next, we showed that RecJ is required for the RecET-mediated illegitimate recombination with or without UV irradiation. The *recJ* gene encodes a 5′-to-3′ single-stranded-DNA-specific exonuclease (17). In homologous recombination, it has been proposed that RecJ processes double-stranded ends of DNA in association with the RecQ helicase to produce 3′ single-stranded DNA ends (5). Ukita and Ikeda previously showed that RecJ also plays a role in UV-induced illegitimate recombination (23). In this study, we indicated that both 5′ exonucleases, RecE and RecJ, are required for the RecET-mediated illegitimate recombination. If we assume that a broken end has a long 5′ single-stranded tail, then RecJ may be required for digestion of a 5′ single strand, resulting in the formation of a blunt end. Therefore, the role of RecJ in RecET-mediated illegitimate recombination may be in the preprocessing of DNA with long 5′ single-stranded tails. It should be noted that illegitimate recombination at some hotspots is diminished by the defect of the RecJ function. The preprocessing of DNA ends by RecJ may be required for illegitimate recombination at these hotspots.

Next, we found that RecO and RecR are required for RecET-mediated illegitimate recombination with or without UV irradiation. In homologous recombination, RecO and RecR facilitate binding of the RecA protein to single-stranded DNA that is bound by SSB, thus promoting homologous recombination (3). However, our results showed that RecA is not required for RecET-mediated illegitimate recombination under UV-irradiated conditions. Therefore, the roles of RecO and RecR in illegitimate recombination may be different from those in homologous recombination. Since the RecO protein promotes renaturation of complementary single-stranded DNA (18), a role for RecO in illegitimate recombination may be to facilitate the annealing of complementary single-stranded DNAs, forming a hydrogen-bonded intermediate of illegitimate recombination. The fact that short regions of homology are required for RecET-mediated illegitimate recombination is consistent with the proposed role of RecO. The role of RecR in illegitimate recombination may be to help the function of RecO through association with it.

Consistent with the previous results (9), we showed here that RecQ suppresses the RecET-mediated illegitimate recombination with or without UV irradiation. Based on the 3′-to-5′ DNA helicase activity of RecQ, we interpreted that the RecQ protein may unwind a hydrogen-bonded intermediate of the DNA end-joining region which is formed by the annealing of DNA ends with short homologies, thus exhibiting the suppression of the recombination. RecET-mediated illegitimate recombination may be suppressed by the helicase activity of RecQ in a way similar to the mechanism proposed by Hanada et al. (9).

It should be noted that RecET-mediated illegitimate recombination is independent of the RecA function under UV-irradiated conditions, but it is increased by the *recA* mutation without UV irradiation. Since RecA participates in major recombination repair pathways, RecBCD, RecE, and RecF, the defect of these pathways may result in the accumulation of

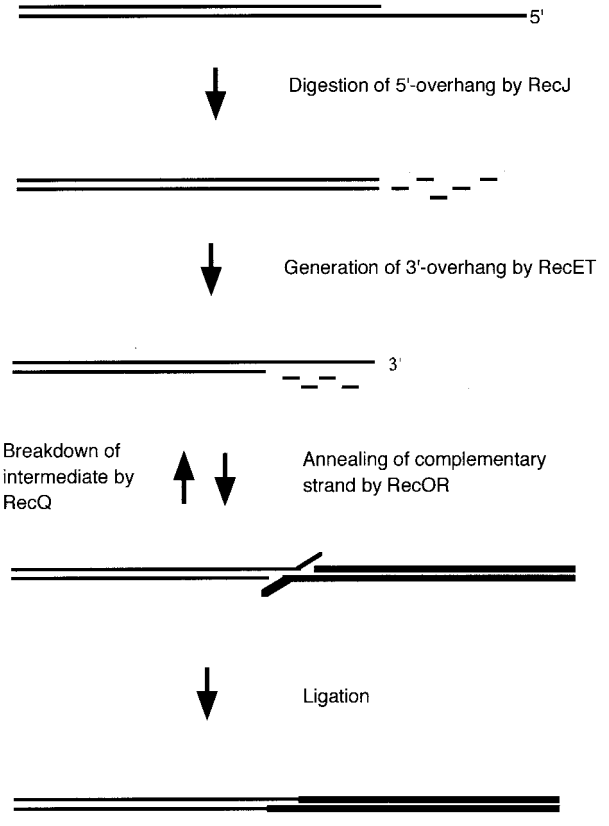


FIG. 2. Model for RecET-mediated illegitimate recombination. RecJ digests a 5′ single-strand overhang, forming the blunt end. RecE digests the 5′ single strand of the blunt end, producing a 3′ single-strand overhang. RecT protects the 3′ single-strand overhang from digestion by ExoI. RecOR promotes annealing of complementary single-stranded DNAs, forming a hydrogen-bonded intermediate and resulting in a recombinant molecule through ligation. RecQ suppresses the formation of the hydrogen-bonded intermediate by unwinding it.

unrepaired DNA ends, which may enhance spontaneous illegitimate recombination mediated by RecET. Further study is needed to clarify this problem.

Finally, we propose a model for RecET-mediated illegitimate recombination, shown in Fig. 2. DNA ends produced by DNA double-strand breaks may be mostly a long 5′ single-strand overhang. RecJ may digest this 5′ single strand, forming the blunt end. RecE may digest the 5′ single strand of the blunt end, producing a 3′ single-strand overhang. RecT may protect the 3′ single-strand overhang from digestion by ExoI. RecOR may promote annealing of complementary single-stranded DNAs, forming a hydrogen-bonded intermediate. RecQ may suppress the formation of the hydrogen-bonded intermediate by unwinding it. The roles of RecF and RuvC, which are known to participate in the RecE recombination repair pathway (6, 16), in illegitimate recombination should be clarified in the near future.

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