The role of UvrD in RecET-mediated illegitimate recombination in *Escherichia coli*

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(Received 23 May 2006, accepted 18 July 2006)

To study the mechanism of RecET-mediated illegitimate recombination, we examined the formation of λ bio-transducing phage in *Escherichia coli* in the presence or absence of UV irradiation. We have previously reported that coexpression of RecE and RecT enhances the frequency of *recA*-independent illegitimate recombination. RecJOR proteins are required for this RecET-mediated illegitimate recombination, and RecQ suppresses it. Here, we showed that the frequencies of both spontaneous and UV-induced RecET-mediated illegitimate recombination events are reduced by a *uvrD* mutation. It should be noted that UvrD is required for illegitimate recombination only in the presence, but not in the absence, of RecET. In contrast, frequencies of RecET-mediated illegitimate recombination were not affected by *ruvAB*, *ruvC*, *recG*, and *recN* mutations. The frequency of spontaneous and UV-induced illegitimate recombination in the *uvrD recR* double mutant was comparable to that of the *uvrD* single mutant, suggesting that UvrD works at the same step as RecR in the RecET-mediated recombination pathway. Nucleotide sequence analyses of the recombination junctions showed that RecET-mediated illegitimate recombination detected in UvrD-deficient strain is short-homology-dependent. Based on these and previous results, we propose a model for the role of UvrD on RecET-mediated illegitimate recombination.

Key words: UvrD, RecET, illegitimate recombination, short-homology-dependent, *recA*-independent

In *Escherichia coli*, the main homologous recombination pathway requires the functions of the RecA and RecBCD proteins. The recombination and repair defects in *recB recC* mutants can be suppressed by two kinds of mutations called *sbcA* and *sbcB* (Lloyd and Buckman, 1985; Templin et al., 1972). The *sbcA* mutations induce expression of RecE and RecT proteins, which mediate homologous recombination through the RecE recombination pathway (Clark et al., 1993). This pathway also requires RecA, RecF, RecJ, RecO, RecR, RuvA, and UvrD (Gillen et al., 1981; Kuzminov, 1999; Luisi-DeLuca et al., 1989; Mendonca et al., 1993). The RecJ exonuclease and RecQ helicase are thought to process the double-stranded ends of DNA to produce 3' single-stranded DNA ends

(Clark and Sandler, 1994; Lovett and Kolodner, 1989). RecO promotes annealing of complementary single-stranded DNA ends and strand exchange in RecA-mediated homologous recombination (Luisi-DeLuca and Kolodner, 1994; Umezumi and Kolodner, 1994). RecR protein, which is known to interact with RecO protein, enhances RecO protein's ability to stimulate displacement of SSB protein from ssDNA by RecA protein (Kantake et al., 2002). UvrD helicase is also thought to promote the generation of a 3' overhang by exonucleases (Matson et al., 1994).

Chromosomal aberrations are caused primarily by illegitimate recombination, which includes duplication, deletion, insertion, and translocation events. Illegitimate recombination occurs spontaneously at a low frequency, but it is greatly enhanced by treatment with UV light or other DNA damaging agents (Ikeda et al., 1995). These

Edited by Hideo Shinagawa

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observations indicate that DNA lesions introduced by UV irradiation cause illegitimate recombination. There are two types of illegitimate recombination that lead to the formation of λ bio-transducing phage: short-homology-dependent illegitimate recombination (SHDIR) and short-homology-independent illegitimate recombination (SHIIR). SHIIR occurs between sequences with virtually no homology and is mediated by DNA topoisomerases I or DNA gyrase (Shimizu et al., 1997; Ashizawa et al., 1999). SHDIR is induced by UV irradiation or other DNA-damaging agents and requires short regions of homology between recombination sites (Hanada et al., 2000; Shiraishi et al., 2005).

In SHDIR, the RecJ exonuclease promotes recombination, but RecQ helicase suppresses it (Ukita and Ikeda, 1996; Hanada et al., 1997). To explain roles of RecJQ in SHDIR, we have previously proposed a model of illegitimate recombination, the double-strand break-and-join model (Yamaguchi et al., 1995; Ukita and Ikeda, 1996; Hanada et al., 1997). In this model, RecJ may modify 5' protruding ends, which are produced by DNA double-strand breaks (DSBs), to form blunt ends. RecQ DNA helicase may disrupt recombination intermediates formed by the annealing of DNA ends before the ligation step. This hypothesis is supported by the observation that RecQ helicase disrupts structures such as joint molecules or four-way junctions (Harmon and Kowalczykowski, 1998).

In recent studies, we found that coexpression of RecE and RecT enhances the frequency of SHDIR (Yamaguchi et al., 2000; Shiraishi et al., 2002). 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 (Joseph and Kolodner, 1983a; Joseph and Kolodner, 1983b). A model has been proposed in which this exonuclease produces 3' single-strand at DNA ends, making them suitable for DNA-end-joining (Keim and Lark, 1990). RecT promotes annealing of complementary single DNA strands and can mediate ligation of the molecules (Hall et al., 1993; Hall and Kolodner, 1994). This model is consistent with our finding that RecET is involved in SHDIR. Furthermore, overexpression of ExoI suppresses this RecET-mediated illegitimate recombination (Yamaguchi et al., 2000). In addition, we found that RecJ, RecO, and RecR are required for the RecET-mediated illegitimate recombination, but RecA function is not (Shiraishi et al., 2002). We also found that RecQ plays a role in suppression of this recombination (Shiraishi et al., 2002).

The frequencies of illegitimate recombination during thermal induction of *E. coli* λ cI857 *ind*⁻ lysogens were measured using the λ Spi⁻ phage assay (Ikeda et al., 1995). Specialized transducing phage generated from the λ prophage by illegitimate recombination usually contains the *E. coli* gene *gal* or *bio*, which is adjacent to the

phage genome. Most of the transducing phages are defective in the *red-gam* region of their genome and can form plaques on an *E. coli* P2 lysogen lawn (Spi⁻ phenotype), whereas normal λ phages cannot. Thus, it is possible to select λ Spi⁻ phages from the phage pool. The number of λ Spi⁻ phages is assumed to be the same as that of λ bio transducing phages, since previous experiments have shown that most λ Spi⁻ phages are λ bio phages (Ikeda et al., 1995; Yamaguchi et al., 1995).

Since an *E. coli* K-12 strain, which was used as the wild-type, does not contain the *recE* and *recT* genes, we introduced a plasmid pRAC3, which carries the *recE* and *recT* genes, into the wild-type and mutant strains (Table 1). Unless otherwise indicated, we refer to the pRAC3-transformed strains simply as the wild-type and mutant strains. The C-terminal domain of the *recE* gene and the full length of the *recT* gene are contained in pRAC3 (Luisi-DeLuca et al., 1988), but it maintains the functions that promote homologous recombination and illegitimate recombination (Takahashi et al., 1993; Yamaguchi et al., 2000).

To examine roles of Rec or Uvr functions in RecET-mediated illegitimate recombination, we measured the frequency of λ Spi⁻ phage production in *recG*, *recN*, *ruvAB*, *ruvC* or *uvrD* mutants carrying pRAC3. The frequency of both spontaneous and UV-induced λ Spi⁻ phage production was reduced in the *uvrD*-deficient strains compared to the wild-type strain (Table 2). On the other hands, frequencies of both spontaneous and UV-induced λ Spi⁻ phage production in the *recG*, *recN*, *ruvAB* and *ruvC* mutants were comparable to that of the wild-type strain (data not shown). It should be noted that UvrD is required for RecET-mediated illegitimate recombination, but not for illegitimate recombination in the absence of RecET.

In addition, we introduced the *uvrD* mutation into the *recR* mutant, and we measured the frequency of illegitimate recombination in the *uvrD recR* double mutant. Frequency of spontaneous as well as UV-induced illegitimate recombination in the *uvrD recR* double mutant was comparable to that of the *uvrD* single mutant (data not shown). These results suggested that UvrD works at the same step as RecR in the RecET-mediated recombination pathway.

Next, we determined the distribution of recombination junctions in λ Spi⁻ phage isolated from the RecET-expressing *uvrD*-deficient strain under UV-irradiation. Since illegitimate recombination takes place between *E. coli* *bio-uvrB* 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. We have previously reported that, in the phages derived from the RecET-expressing wild-type strain (HI2899) under UV-irradiation, most of the recombination takes place at one of the

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

Strains	Description	Source or construction
Ymel	<i>supE supF</i>	Our collection
WL95	<i>supE supF metB trpR hsdR tonA</i> (P2)	Our collection
HI2898	594 derivative, <i>sup</i> ⁰ (λ <i>cI857</i> ^a)/pBR322	HI1162
HI2899	594 derivative, <i>sup</i> ⁰ (λ <i>cI857</i> ^a)/pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺) ^b	HI1162
HI3155	594 derivative, <i>sup</i> ⁰ <i>recG258::Tn5</i> (λ <i>cI857</i> ^a)/pBR322	HI3154
HI3156	594 derivative, <i>sup</i> ⁰ <i>recG258::Tn5</i> (λ <i>cI857</i> ^a)/pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺) ^b	HI3154
HI3121	594 derivative, <i>sup</i> ⁰ <i>recN1502::Tn5</i> (λ <i>cI857</i> ^a)/pBR322	HI3120
HI3122	594 derivative, <i>sup</i> ⁰ <i>recN1502::Tn5</i> (λ <i>cI857</i> ^a)/pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺) ^b	HI3120
HI3087	594 derivative, <i>sup</i> ⁰ Δ <i>ruvAB200::kan</i> (λ <i>cI857</i> ^a)/pBR322	HI2770
HI3088	594 derivative, <i>sup</i> ⁰ Δ <i>ruvAB200::kan</i> (λ <i>cI857</i> ^a)/pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺) ^b	HI2770
HI3089	594 derivative, <i>sup</i> ⁰ Δ <i>ruvC200::kan</i> (λ <i>cI857</i> ^a)/pBR322	HI2771
HI3090	594 derivative, <i>sup</i> ⁰ Δ <i>ruvC200::kan</i> (λ <i>cI857</i> ^a)/pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺) ^b	HI2771
HI3208	AB1157 derivative, (λ <i>cI857</i> ^a)/pBR322	HI2051
HI3209	AB1157 derivative, (λ <i>cI857</i> ^a)/pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺) ^b	HI2051
HI3238	AB1157 derivative, Δ <i>uvrD::tet</i> (λ <i>cI857</i> ^a)/pBR322	HI1521 $\times$ λ <i>cI857</i>
HI3239	AB1157 derivative, Δ <i>uvrD::tet</i> (λ <i>cI857</i> ^a)/pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺) ^b	HI1521 $\times$ λ <i>cI857</i>
HI3276	AB1157 derivative, Δ <i>uvrD::tet</i> <i>recR252::Tn/Ominikan</i> (λ <i>cI857</i> ^a)/pBR322	HI3261
HI3277	AB1157 derivative, Δ <i>uvrD::tet</i> <i>recR252::Tn/Ominikan</i> (λ <i>cI857</i> ^a)/pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺) ^b	HI3261

^a All λ *cI857 ind*⁻ lysogens contain one unit of λ prophage.^b Plasmid pRAC3 is a pBR322 plasmid containing *E. coli recE*⁺*T*⁺ genes (Takahashi et al., 1993)Table 2. Effect of the *uvrD* mutation on spontaneous or UV-induced RecET-mediated illegitimate recombination

UV dose (J/m ²)	Strain ^a	Relevant mutation	Plasmid	Spi ⁻ phages (10 ⁻⁸) per total λ phage (SEM) ^b		Frequency relative to control	Burst size ^c (SEM)
0	HI3208	wild type	pBR322	0.13	(0.035)	1	120 (37)
0	HI3238	Δ <i>uvrD::tet</i>	pBR322	0.14	(0.025)	1.1	72 (19)
0	HI3209	wild type	pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺)	2.2	(0.95)	17	110 (25)
0	HI3239	Δ <i>uvrD::tet</i>	pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺)	0.17	(0.007)	1.3	65 (15)
10	HI3208	wild type	pBR322	2.9	(0.88)	1	115 (23)
10	HI3238	Δ <i>uvrD::tet</i>	pBR322	2.2	(1.2)	0.76	62 (20)
10	HI3209	wild type	pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺)	25	(9)	8.6	105 (19)
10	HI3239	Δ <i>uvrD::tet</i>	pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺)	1.4	(0.72)	0.48	55 (18)
20	HI3208	wild type	pBR322	4.0	(0.95)	1	112 (15)
20	HI3238	Δ <i>uvrD::tet</i>	pBR322	3.2	(1.6)	0.8	47 (15)
20	HI3209	wild type	pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺)	47	(2)	12	102 (13)
20	HI3239	Δ <i>uvrD::tet</i>	pRAC3 (<i>recE</i> ⁺ <i>T</i> ⁺)	3.2	(1.7)	0.8	49 (18)

^a All strains are *E. coli* K-12 AB1157 or its derivatives containing one unit of λ *cI857 ind*⁻ prophage. *E. coli* λ *cI857 ind*⁻ lysogen or its derivatives was grown to 1×10^8 cells/mL and irradiated with UV at several dose. The lysogen was induced by incubation at 42°C for 15 min with shaking and then incubated at 37°C for 2 hr. 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 (Ikeda et al., 1995).^b All numbers are averages of four determinations.^c Burst size indicates the total number of phages per cell.

Fig. 1. Nucleotide sequences of the recombination junctions of *λbio* transducing phages isolated from RecET-expressing and RecET-non-expressing strains under UV irradiation. The distributions of recombination sites of recombinant phages derived from various strains were determined by PCR. The junctions were classified into four classes: hotspot I, II, III, and non-hotspot. These junctions were sequenced with an ABI DNA sequencer. (A) Schematic representation of the recombination sites in this assay. (B, Panel a) Sequences of hotspot I detected at the recombination junctions of *λbio* transducing phages URA23, which were isolated from the RecET-expressing HI3239 *uvrD* mutants. Phages UPV41 were isolated from the RecET-non-expressing HI3238 *uvrD* mutants. Homologous sequences are shown in bold. Map coordinates for phage and bacterial sequences are indicated. (Panel b) Sequences of hotspot II detected at the recombination junctions of *λbio* transducing phages URA12, which were isolated from the RecET-expressing *uvrD* mutants. Phages UPV19 were isolated from the RecET-non-expressing *uvrD* mutants. (Panel c) Sequences of hotspot III detected in the recombination junctions of *λbio* transducing phages UPV33 and UPV56, which were isolated from the RecET-non-expressing *uvrD* mutant. (Panels d and e) Sequences of non-hotspot sites detected in the recombination junctions of *λbio* transducing phages derived from the RecET-non-expressing *uvrD* mutant (HI3238). (Panels f to h) Sequences of non-hotspot sites detected in the recombination junctions of *λbio* transducing phages derived from the RecET-expressing *uvrD* mutant (HI3239).

three hotspots. The relative rates of recombination at hotspot I, II, and III were 46%, 4%, and 34%, respectively, indicating that RecET-mediated illegitimate recombina-

tion is short-homology-dependent (Shiraishi et al., 2002). In this study, we tested the recombination junctions of 60 *λ* Spi⁻ phages derived from the RecET-expressing *uvrD*-

deficient strain (HI3239) under UV-irradiation and found that the relative rates of recombination at hotspots I, II, and III were 72%, 10%, and 0%, respectively (Table 3). The rate of recombination at hotspots III with the RecET-expressing *uvrD*-deficient strain (HI3239), was significantly low, compared with that with the RecET-expressing wild-type strain (HI2899). On the other hand, the rate of recombination at hotspot III with the RecET-non-expressing *uvrD*-deficient strain (HI3238) was comparable with that with the RecET-non-expressing wild-type strain (HI2898) (Table 3). It is therefore suggested that UvrD is required for recombination at hotspot III with the RecET-expressing strain. Reason why recombination at hotspot III does not occur in the *uvrD*-deficient strain has not been known yet.

The junctions of the *λbio* transducing phages UPV41 and URA23 were produced by recombination at hotspot I, which was previously found by Yamaguchi et al. (Yamaguchi et al., 1995). At this hotspot, the recombination sites are known to share a 9-bp region of homology, as shown in Fig. 1B, panel a. The hotspot sites II and III share 13 bp and 5 bp regions of homology, respectively, as shown in Fig. 1B, panel b and c. Panels d and e in Fig. 1B show the junction sequences of *λbio* transducing phages derived from recombination at non-hotspot sites in the RecET-non-expressing *uvrD*-deficient strain (average length of homology, 9.3 bp), and panels f to h show the junction sequences of *λbio* transducing phages derived from recombination at non-hotspot sites in the RecET-expressing *uvrD*-deficient strain (average length of homology, 9.5 bp). All recombination sites shared short regions of homology between *E. coli* and *λ* phage DNA. These results indicate that short regions of homology also have a role in illegitimate recombination in the absence of UvrD function.

Early studies of illegitimate recombination suggested that 3' single-stranded overhangs were required to promote the recombination (Allgood and Silhavy, 1991; Yamaguchi et al., 2000). Here, we have shown that UvrD helicase is required for RecET-mediated illegitimate recombination, but not for illegitimate recombination in the absence of RecET. The UvrD protein has a

3'→5' DNA helicase activity and is suggested to play a role in processing of DNA ends in combination with nucleases (Matson et al., 1994). This is consistent with a model in which UvrD helps the RecE exonuclease to produce 3' single-strand overhangs, thus increasing the frequency of illegitimate recombination.

In homologous recombination, RecO, RecR, and possibly RecF proteins have been shown to promote formation of RecA nucleoprotein filaments on single-stranded DNA (ssDNA) gaps covered with single-stranded DNA binding protein (SSB), suggesting that RecFOR help RecA to overcome inhibition by SSB (Umezumi et al., 1993). In addition, Veaute et al. (2005) suggested that UvrD helicase dismantles RecA nucleoprotein filaments, thus preventing homologous recombination. Namely, RecFOR and UvrD might have adverse roles in homologous recombination. These results raise another possibility that UvrD indirectly enhances the RecET-mediated illegitimate recombination by preventing RecA-mediated homologous recombination. However our present studies showed that the frequency of illegitimate recombination was reduced in the *recR*- or *uvrD*-deficient strains with pRAC3, compared to those in the wild-type strain. Furthermore, the frequency of illegitimate recombination in the *uvrD recR* double mutant with pRAC3 was comparable with those in the wild-type strains. These results suggested that UvrD works at the same step as RecR in the RecET-mediated illegitimate recombination. Therefore the above possibility seems to be unlikely.

We have previously reported that co-expression of RecE and RecT enhances the frequency of spontaneous and UV-induced illegitimate recombination (Shiraishi et al., 2002). Based on these and the previous results, we propose the following model for RecET-mediated illegitimate recombination. DNA double-strand breaks produce DNA ends which have long 5' single-stranded overhangs. RecJ digests these 5' single-strands, forming blunt ends. RecE digests the 5' single-strands of the blunt ends, producing 3' single-stranded overhangs, and UvrD may facilitate the generation of 3' single-stranded overhangs by the RecE exonuclease. These 3' overhangs might bind with SSB and then degraded by Exonuclease I, a single-

Table 3. Summary of distribution of recombination sites of *λbio* transducing phages formed following UV irradiation^a

Strain	Relevant mutation	Plasmid	Number of phages tested	Rate of recombination (Rate with 95% probability) at:			
				HotI (%)	HotII (%)	HotIII (%)	Non-hot (%)
HI2898 ^b	wild type	pBR322	48	33 (19–52)	21 (10–39)	2 (0.2–10)	44 (27–66)
HI2899 ^b	wild type	pRAC3	50	46 (29–69)	4 (0.5–14)	34 (20–54)	16 (7–31)
HI3238	<i>uvrD</i>	pBR322	60	75 (54–95)	3 (0.3–12)	5 (0.6–16)	17 (8–32)
HI3239	<i>uvrD</i>	pRAC3	60	72 (51–96)	10 (3.7–21)	0 (0.1–3.7)	18 (9–33)

^a The distribution of recombination sites of recombinant phages derived from the *uvrD* strain was determined by PCR. The junctions were classified into four classes: hotspot I (Hot I), II (Hot II), III (Hot III), and non-hotspot (Non-hot). The dose of UV irradiation was 20 J/m².

^b The data with HI2898 and HI2899 were cited from Shiraishi et al. (2002).

stranded DNA-specific nuclease, whose activity is stimulated by SSB binding to ssDNA (Molineux and Geftter, 1975). A role of RecT might be to compete with SSB for binding to the 3' single-stranded overhangs, thus protecting them from degradation by ExoI. In addition, RecO and RecR are known to bind to SSB-coated ssDNA without displacing SSB (Umez and Kolodner, 1994). A possible role of RecOR could also be the protection of the 3' single-stranded overhangs from digestion by ExoI. Since both RecT and RecO are known to have annealing activities of ssDNA, these proteins may promote annealing of complementary ssDNA, forming hydrogen-bonded intermediates. Finally DNA ligase would play a role in joining of the hydrogen-bonded intermediates.

We thank Dr. S. W. Matson for providing *E. coli* strains and Dr. K. Egawa for encouragement. This study was supported by Grants-in-Aid for Scientific Research on Priority Areas (B) to Y.O. from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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