
EXPERIMENTAL WORKS

Features of DNA Helicase Encoded by the *uvrD* Gene of *Deinococcus radiodurans* R1 in *Escherichia coli* K-12 Cells

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Received August 28, 2018; revised September 18, 2018; accepted December 12, 2018

Abstract—Reparative helicase II of *Deinococcus radiodurans* performs an unexpected critical function in repair of double-strand DNA breaks through the mechanism of extended synthesis-dependent strand annealing (ESDSA), while it is considered as an optional participant in the RecF pathway of recombinational repair in *Escherichia coli*. A fragment of genomic DNA of the radioresistant bacterium *Deinococcus radiodurans* with the *uvrD* gene encoding DNA helicase II, which is involved in excision repair of nucleotides, mismatch repair, and recombinational repair and replication, was cloned in the cells of the model object, *Escherichia coli* K-12. The pCR 2.1-*uvrD*⁺ plasmid restores resistance to ultraviolet light of mutant cells of *Escherichia coli uvrD*⁻, *helD*⁻, and *rep*⁻ defective in reparative helicase II, helicase IV, and replicative helicase Rep, respectively, almost to the level of wild-type AB1157 and *uvrD*⁺, and, to a lesser extent, the strain with a mutation in the *recQ* gene encoding the key helicase of recombinational repair RecQ. The protective effect is also noticeable when strains with the plasmid are irradiated with γ -rays. It is established that *Deinococcus radiodurans* UvrD helicase possesses broad possibilities.

Keywords: DNA helicase II, *Deinococcus radiodurans*, *uvrD*, *rep*, *recQ*, *helD*, functional complementation, radiation resistance

DOI: 10.3103/S0891416820010048

INTRODUCTION

The *uvrD* gene encodes helicase II, which plays an important role in nucleotide excision repair (NER), correction of incorrectly paired bases, and DNA replication in prokaryotes [1]. It is also known that it participates in repair of gaps in DNA through the mechanism of RecFOR-dependent recombinational repair [2]. At the same time, its contribution to recombinational DNA repair can be underestimated due to the presence of the active RecBCD-dependent pathway of recombinational repair in *Escherichia coli*. In the extremely radioresistant bacterium *Deinococcus radiodurans*, only the RecFOR pathway for recombinational repair functions [3], and the contribution of helicase II to repair of single-strand gaps, as well as the most dangerous forms of damage to DNA, double-strand breaks (DSBs), is decisive, against this background.

The *D. radiodurans* bacterium is well known due to its high resistance to extreme effects of drying and radiation that cause various types of DNA damage, including DSBs. Since a single unrepaired DSB of DNA is usually lethal, DSBs are considered very severe forms of genome damage. The extreme radioresistance of the deinococcus is associated with its ability to reconstruct the genome from hundreds of chro-

mosomal fragments. The genome reconstruction proceeds through a two-stage process. Stage 1 is extended synthesis-dependent strand annealing (ESDSA), which collects fragments of the genome into long linear intermediates. Stage 2 is recombination that generates ring chromosomes [4, 5]. *D. radiodurans* has homologues of key components of the RecF recombination pathway [3], the main pathway of recombinational repair in this organism, as is the case in other bacteria that do not have homologues of the RecBCD recombination pathway.

In the model *E. coli* bacterium, the RecF pathway includes 5'–3'-single-stranded-DNA-specific exonuclease RecJ, helicase RecQ (*ecRecQ*), and proteins RecF, RecO, and RecR, which act together to provide the loading of the RecA protein onto single-stranded DNA [2]. Helicases UvrD (*ecUvrD*) and HelD (*ecHelD*) are considered possible, but not necessary, participants in this recombination pathway [6], although the role of the UvrD helicase in repair of replicative forks under Rep (*ecRep*) helicase damage has been recognized [7]. However, in *D. radiodurans*, the UvrD (*drUvrD*) reparative helicase performs an unexpected critical function in repair of DNA DSBs by the ESDSA mechanism [3]. It has been suggested that UvrD, replacing RecQ, acts in tandem with RecJ and

Table 1. Characteristics of used bacterial strains

Strain	Genotype	Source/method of obtaining
<i>E. coli</i> K-12 AB1157	Wild type (<i>F⁻ ara14 galK2 his-4 proA2 argE3 thi1 tsx33 thr1 leu6 lacY1 mtl1 xyl5 rpsL31 supE37</i>)	Laboratory collection
HA1159	<i>uvrD</i> ₂₁₀	Laboratory collection
BL1192	As AB1157, but <i>helD1021::Tn10-9</i>	Phage P1 transduction
IT1110	<i>rep71</i>	Laboratory collection
KD1996	<i>recQ61::Tn3</i>	Laboratory collection
Z905	Δ <i>recA</i>	Laboratory collection
<i>D. radiodurans</i> R1	Wild type	Collection of the Institute of Biochemistry and Physiology of Microorganisms of the Russian Academy of Sciences

generates single-stranded tails, on which the RecFOR complex will stimulate the loading of RecA [3]. The question remains intriguing of what ultimately underlies the high radioresistance of *D. radiodurans*. A promising direction in studying the mechanisms of radioresistance is the transfer of the *uvrD* gene from *D. radiodurans* to the genetic background of *E. coli* to compare the properties of this reparative helicase in species with different radioresistance.

The aim of this work was to clone the UvrD reparative helicase of the bacterium *D. radiodurans* into *E. coli* cells and to study its ability to complement mutations in various helicases and restore the radio-stability of this microorganism.

MATERIALS AND METHODS

Bacterial strains. The characteristics of the strains used are presented in Table 1.

Plasmids. The plasmid pCR 2.1-TOPO (Amp^r Kan^r/Neo^r 5 kb) (Invitrogen, 1997) was used in the work. The plasmid carries determinants of Ap^r resistance to ampicillin and Km^r resistance to kanamycin.

Oligonucleotides. For cloning of *D. radiodurans uvrD*, *uvrD*₂₁₀-F 5'-AAGAAGCGTCCGACCAAGTG and *uvrD*₂₁₀-R 5'-TCGTCATCCGGAGCTGAGTC primers were used. For cloning of *uvrD E. coli*, *uvrD*₂₁₀-F 5'-CATCTCTGACCTCGCTGATA, *uvrD*₂₁₀-R 5'-AACGTTACACCGACTCCAGC primers were used (Syntol, Moscow).

Media. For UV and γ -irradiation of cells, M9 medium containing 0.05 M MgSO₄, Na₂HPO₄ (6 g), KH₂RO₄ (3 g), NaCl (0.5 g), NH₄Cl (1 g) per 1 L of water (pH 7.2) was used. For cell growth, AP broth, an aminopeptide (Samson), diluted twice with 0.15 M NaCl, was used. Cell titration was carried out on AP agar (AP broth containing 1.6% agar). Ampicillin was added to the media at a concentration of 50 μ g/mL, and kanamycin was added at a concentration of 40 μ g/mL.

Isolation of plasmid DNA. DNA was isolated by the standard alkaline lysis [8].

Transformation of *E. coli* cells. *E. coli* cells were transformed on the basis of the resistance to ampicillin or kanamycin with isolated recombinant plasmids pCR 2.1-UvrD by the calcium method using the standard protocol [8] followed by plating of the samples on AP agar with ampicillin or kanamycin, respectively.

Study of cell survival dependence on UV and γ -radiation dose. *E. coli* cells grown on AP broth until the middle of the exponential growth phase (optical density of 0.2–0.4 at 630 nm) were pelleted by centrifugation (10000 rpm, 10 min) and resuspended in the initial volume of M9 medium. Irradiation was carried out at room temperature in glass Petri dishes on a Khromoskop device for UV irradiation at the radiation power of \sim 450 mJ/m²/s in the range of 200–280 nm, selecting 0.1 mL aliquots of the culture. γ -irradiation was carried out on an Issledovatel' installation containing ⁶⁰Co with a dose rate of 200 Gy/min. The irradiated cells were plated on AP agar after appropriate dilution with M9 medium, and the number of colonies was counted after 24 h. UV irradiation, cultivation, and plating were performed under red light conditions to avoid photoreactivation. With each of the used strains, three to six independent experiments were performed.

Mathematical processing of experimental results. The processing of the data on the dependence of *S* on the radiation dose (*D*) and plotting the survival curves $\log S = f(D)$ were performed by the least squares method using the Origin 2016 program. The $\log S = f(D)$ curves were linearly approximated as $\log S = A + BD$. The plots show mean values and standard error. In Table 2, the values of parameters *A* and *B*, which characterize the extrapolation number and the slope of the curve, are presented. To compare the radiosensitivity, the dose modification factor (DMF) was determined as the ratio of parameters *B* for pairs : strain without the plasmid/strain with the plasmid.

Table 2. Comparison of the sensitivity of *E. coli* strains carrying the plasmid with the cloned *uvrD* gene of *D. radiodurans* to UV light and ionizing radiation

strain	UV light			γ -radiation		
	A	$-B$, m ² /J	DMF	A	$-B$, kGy ⁻¹	DMF
<i>uvrD</i> *	-0.10 ± 0.50	0.081 ± 0.015		-0.11 ± 0.16	7.7 ± 0.4	
<i>uvrD</i> /pCR 2.1- <i>uvrD</i> ⁺	-0.80 ± 0.22	0.019 ± 0.001	4.3	-0.40 ± 0.10	4.5 ± 0.2	1.7
<i>recQ</i>	0.53 ± 0.08	0.079 ± 0.003		-0.08 ± 0.38	10.4 ± 0.8	
<i>recQ</i> /pCR 2.1- <i>uvrD</i> ⁺	0.27 ± 0.12	0.042 ± 0.001	1.9	0.50 ± 0.02	8.1 ± 0.1	1.3
<i>helD</i>	0.40 ± 0.02	0.051 ± 0.001		0.51 ± 0.19	10.4 ± 0.4	
<i>helD</i> /pCR 2.1- <i>uvrD</i> ⁺	0.53 ± 0.09	0.039 ± 0.002	1.3	-0.31 ± 0.25	7.1 ± 0.7	1.5
<i>rep71</i>	0.11 ± 0.38	0.053 ± 0.006		0.52 ± 0.37	6.0 ± 1.0	
<i>rep71</i> /pCR 2.1- <i>uvrD</i> ⁺	0.18 ± 0.06	0.024 ± 0.001	2.2	0.59 ± 0.55	6.7 ± 1.2	0.9
AB1157	0.57 ± 0.07	0.025 ± 0.001		0.02 ± 0.12	3.2 ± 0.3	
AB1157/pCR 2.1- <i>uvrD</i> ⁺	0.77 ± 0.32	0.028 ± 0.003	0.9	-0.04 ± 0.18	3.0 ± 0.3	1.1
<i>uvrD</i> /pCR 2.1- <i>uvrD</i> _{Ec} ⁺	-0.30 ± 0.08	0.021 ± 0.001	3.9	-0.32 ± 0.15	2.7 ± 0.3	2.9
<i>uvrD</i> /pCR 2.1- <i>uvrD</i> ^{-*}	-1.45 ± 0.30	0.051 ± 0.005	1.6	—	—	

* The curve was L-shaped.

RESULTS AND DISCUSSION

The full-length copy of the *uvrD* gene of *D. radiodurans* with the supposed promoter was cloned on the pCR 2.1-TOPO plasmid. Reproduction of the plasmid was performed in the *recA* strain defective in recombination. Due to the fact that no consensus promoter for *E. coli* was found in the 5' region, we tested several clones to select the variant with the orientation necessary for expression. The orientation was confirmed by restriction analysis and sequencing. Two such clones with different orientation of the insertion were designated as pCR 2.1-*uvrD*⁺ and pCR 2.1-*uvrD*⁻. The *E. coli uvrD* strain, which is defective in the activity of DNA helicase II, was transformed with both plasmids. Survival curves of the corresponding transformants are presented in Fig. 1, and the parameters of the curves are given in Table 2. The pCR 2.1-*uvrD*⁺ plasmid significantly restored the radioresistance of the *uvrD* recipient, especially in the high-dose region (see Fig. 1, curve 2). In this case, the radioresistance increased in approximately 4.3 times when evaluated according to the DMF parameter. At the same time, the pCR 2.1-*uvrD*⁻ plasmid did not significantly affect the radiosensitivity of transformed cells (see Fig. 1, curve 12). The gene dose apparently does not matter since the pCR 2.1-*uvrD*⁺ plasmid did not affect the UV sensitivity of wild-type cells (see Fig. 1, curve 10). The plasmid with the cloned *E. coli uvrD* gene of was designated as pCR 2.1-*uvrD*_{Ec}⁺. It completely compensated the defect in the *uvrD* gene as expected (see Fig. 1, curve 11). Thus, despite the fact that the UvrD helicase of *D. radiodurans* originates from an unrelated

species, it is able to compensate the defect in the homologous enzyme of *E. coli*.

To determine the ability of the *drUvrD* helicase to compensate defects in other helicases involved in recombination, repair, and replication in *E. coli*, we transformed *E. coli* strains defective in these enzymes with the pCR 2.1-*uvrD*⁺ plasmid. They were used in experiments on the determination of the UV sensitivity. It turned out that the pCR 2.1-*uvrD*⁺ plasmid can compensate the defect not only in helicase II, but to different extents in other helicases as well—HelD, Rep, and RecQ, increasing the survival rate by 1.3, 2.2, and 1.9 times, respectively (see Fig. 1, Table 2). The pCR 2.1-*uvrD*_{Ec}⁺ plasmid did not affect the radioresistance of *helD*, *rep*, and *recQ* mutants (data not shown).

The greatest effect was observed in the *rep*/pCR 2.1-*uvrD*⁺ transformant, which became statistically close in terms of resistance to the wild type (see Fig. 1, curve 8).

Experiments on the radiosensitivity determination were also carried out using γ -rays (Fig. 2, Table 2). The protective effect of the pCR 2.1-*uvrD*⁺ plasmid was less pronounced and was absent in the case of the *rep* mutant, which initially turned out to be sufficiently radioresistant to γ -rays. The observed differences may be associated with a different spectrum of DNA damage after UV and γ -irradiation.

To explain the recorded effect of the *drUvrD* protein presence in *E. coli* cells, the primary sequence of *D. radiodurans* helicase II was aligned with four *E. coli* helicases using the BLASTP program of the National Center for Biotechnological Information (United States). The identity was 35% for the pair *drUvrD/ecUvrD*,

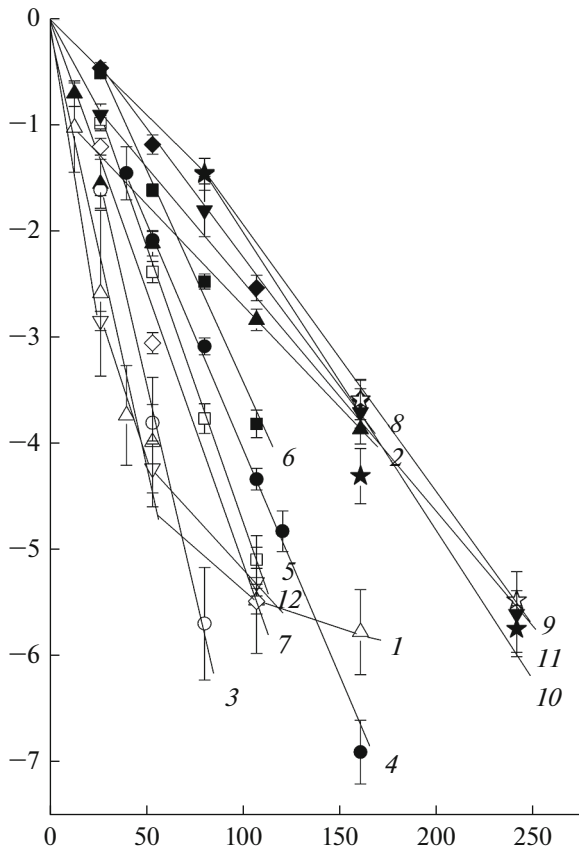


Fig. 1. Lethal effect of UV light on *E. coli* strains. (1) *uvrD* (Δ); (2) *uvrD*/pCR 2.1-*uvrD*⁺ (\blacktriangle); (3) *recQ* (\circ); (4) *recQ*/pCR 2.1-*uvrD*⁺ (\bullet); (5) *helD* (\square); (6) *helD*/pCR 2.1-*uvrD*⁺ (\blacksquare); (7) *rep* (\diamond); (8) *rep*/pCR 2.1-*uvrD*⁺ (\blacklozenge); (9) AB1157 (\star); (10) AB1157/pCR 2.1-*uvrD*⁺ (\blackstar); (11) *uvrD*/pCR 2.1-*uvrD*_{Ec}⁺ (\blacktriangledown); (12) *uvrD*/pCR 2.1-*uvrD*_{Ec}⁻ (∇). Radiation dose (J/m^2) is on the abscissa axis. Survival rate ($\log S$) is on the ordinate axis.

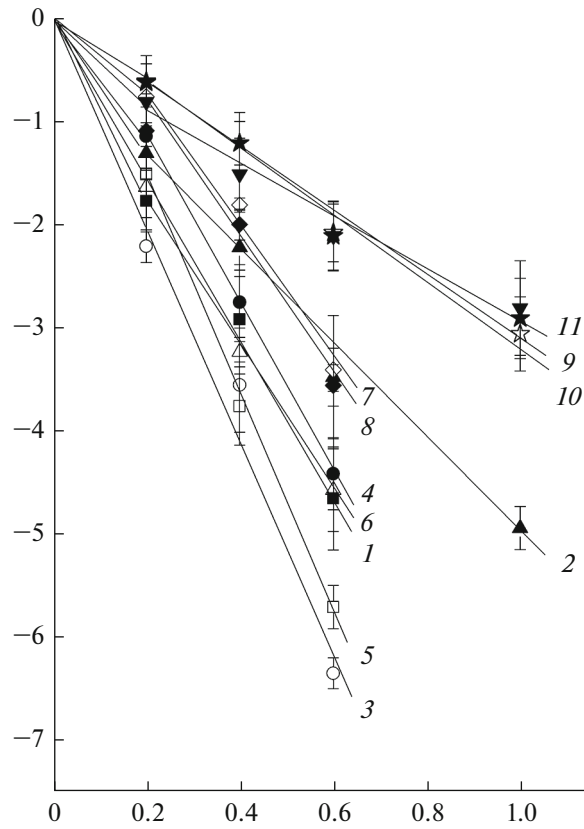


Fig. 2. Lethal effect of γ -radiation on *E. coli* strains. (1) *uvrD* (Δ); (2) *uvrD*/pCR 2.1-*uvrD*⁺ (\blacktriangle); (3) *recQ* (\circ); (4) *recQ*/pCR 2.1-*uvrD*⁺ (\bullet); (5) *helD* (\square); (6) *helD*/pCR 2.1-*uvrD*⁺ (\blacksquare); (7) *rep* (\diamond); (8) *rep*/pCR 2.1-*uvrD*⁺ (\blacklozenge); (9) AB1157 (\star); (10) AB1157/pCR 2.1-*uvrD*⁺ (\blackstar); (11) *uvrD*/pCR 2.1-*uvrD*_{Ec}⁺ (\blacktriangledown). Radiation dose (kGy) is on the abscissa axis, survival rate ($\log S$) is on the ordinate axis.

38% for *drUvrD/ecRep*, and 27% for *drUvrD/ecHelD*. However, typical motifs of helicase II [9], represented by seven elements, were very conservative. They practically coincide in helicases *drUvrD*, *ecUvrD*, *ecRep*, and *ecHelD*. A special case is presented by *ecRecQ* helicase, in which within the entire sequence only one small region at the C-terminus, which is characteristic of all helicases, coincides with *drUvrD*.

Thus, when cloning and studying the properties of *D. radiodurans* UvrD helicase in mutant *E. coli* cells, its ability to compensate defects in a number of helicases involved in various processes, such as repair, replication, and recombination, was established.

The role of the UvrD helicase has been widely characterized in NER and correction of incorrectly paired bases in *E. coli* [10, 11]. It was previously established that inactivation of helicase II significantly reduces the radioresistance of the deinococcus and greatly delays the kinetics of DSB repair [3]. It is

unlikely that the sensitivity to γ -radiation of *D. radiodurans uvrD* cells is associated with a defect in the NER pathway, because mutant bacteria defective in the *uvrA* gene retain survival at the wild-type level after irradiation with ionizing radiation [3]. It is assumed that UvrD is involved in ESDSA, however, in *E. coli*, the RecQ helicase in the complex with the RecJ exonuclease is considered to be the main one on the RecF pathway of recombinational repair [2]. Helicases HelD and UvrD have been considered as alternatives to RecQ [12].

The primary role of DNA helicase II is associated with its ability to bind and untwist specific structures corresponding to intermediates of repair and other processes. From this point of view, the substrate specificity of UvrD is important.

Biochemical studies showed that *D. radiodurans* UvrD is an active DNA-stimulated ATPase that also has ATP-dependent translocase and helicase activities

[13]. In vitro studies showed that *drUvrD* protein translocates along ssDNA in the 3'–5' direction like *ecUvrD* [14], and, despite belonging to the helicase family with 3'–5' polarity, it can untwist duplex DNA in both the 3'–5' and 5'–3' directions [15]. It is interesting that translocase and bipolar helicase activities of *drUvrD* is modulated by the ssDNA-binding protein SSB (which is typical for *E. coli* RecQ [16]). *drUvrD* also exhibits weak helicase activity toward blunt-ended DNA.

These properties of *D. radiodurans* UvrD helicase explain its ability to compensate not only the defect in the *E. coli uvrD* gene, but also in *rep* and *recQ* genes. Rep is a helicase with a preference for the leading DNA strand. It plays an important role in DNA replication [17]. Rep and UvrD are two related helicases of *E. coli*, and inactivation of both is lethal [7]. The contribution of the UvrD helicase to the cell viability is underscored by the fact that the combination of mutations in the *uvrD* gene and the *polA* gene encoding DNA polymerase I is also lethal [18]. The UvrD helicase is capable of displacing oligonucleotides from the synthetic DNA replicative fork in vitro and is necessary for the survival in the absence of the Rep helicase associated with replicative fork processing. It can be assumed that the UvrD helicase can provide regression of replicative forks and facilitate the restart of replicative forks after stopping. The radioprotective effect of *D. radiodurans* UvrD expression against the background of the *E. coli recQ* mutation is very remarkable. In vitro, the RecQ helicase binds and untwists many different DNA substrates [19]. DNA with a single strand tail, DNA with a gap, double-stranded DNA with blunt ends, and covalently closed double-stranded DNA are all untwisted by RecQ. This indicates that this helicase can function on a variety of intermediates involved in DNA metabolism. RecQ is one of the few helicases that do not require a single-stranded region to enter double-stranded DNA [20].

Thus, the substrate specificity of *D. radiodurans* UvrD helicase significantly coincides with the preferences of *E. coli* RecQ helicase. Significant differences in the amino acid sequences of *drUvrD* and *ecRecQ* do not interfere with functional complementation and are explained by the belonging of RecQ to another helicase family SF2 [21]. The contribution of *D. radiodurans* UvrD helicase to the radioresistance restoration of *E. coli rep*, *recQ*, and *helD* mutants can be associated with the replication activation according to the model of DSB recombinational repair associated with the restart of DNA synthesis [22].

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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Translated by D. Novikova