RecO Is Essential for DNA Damage Repair in *Deinococcus radiodurans*

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Deinococcus radiodurans is best known for its astonishing ability to resist the lethal effects of many DNA-damaging agents, including ionizing radiation, UV, hydrogen peroxide, and desiccation (1, 2, 24, 32). This striking feature is due to its extraordinary ability for DNA repair: it can reconstruct a functional chromosome from hundreds of shattered DNA fragments within hours (24). It is believed that RecA-dependent homologous recombination DNA repair plays a vital role in the genome restitution of this organism (3), although DNA repair via RecA-independent pathways is also reported to take part in genome restitution (5, 17, 26, 29, 34).

*Escherichia coli* and many other bacteria possess two RecA-dependent recombinational systems: the RecBCD and RecF pathways (18). These two pathways normally operate independently of each other and have different functions in vivo: RecBCD is the predominant recombination pathway and is responsible for double-strand break repair, while the RecF pathway is involved mainly in the repair of single-stranded gaps. Mutation in the recBC genes results in the sensitivity of *E. coli* cells to various DNA-damaging agents, unless the cells acquire mutations in the sbcA, sbcB, and sbcC/sbcD genes. The RecF pathway becomes active in this situation. Whereas the RecF pathway is encoded more frequently in the bacterial genome (22, 33), suggesting that the RecBCD pathway is absent in this organism (2), whereas the genes coding for the RecF pathway, such as recF, recO, and recR, are present in the genome of several RecF pathway genes in *D. radiodurans* genome (21, 23, 31). These results suggest that DNA repair in this organism occurs mainly via the RecF pathway.

Here we present direct evidence for the vital role of RecO in *Deinococcus radiodurans*’s radioresistance. A recO null mutant was constructed using a deletion replacement method. The mutant exhibited a growth defect and extreme sensitivity to irradiation with gamma rays and UV light. These results suggest that DNA repair agents like mitomycin C but remains resistant to ionizing radiation. Presumably, the RecF pathway plays a substantial role in the reconstitution of the *D. radiodurans* genome and DNA repair. Genetic studies have confirmed the importance of several RecF pathway genes in *D. radiodurans*. The deletion of recN (6) and recQ (10) and the mutation of ruvB (14) result in the increased radiosensitivity of the mutant bacteria. The functions and structures of the pathway proteins RecA (12, 13, 16), RecF (16), RecO (21, 23, 31), RecR (19, 20, 31), and RecQ (9) were also investigated. However, little in vivo evidence has been presented to date for the roles of the unique genes recF, recO, and recR of the RecF pathway in *D. radiodurans*’s radioresistance. Only a recR (15) partial deletion mutant, with a 27-amino-acid substitution at the C-terminal region, was reported to be extremely sensitive to cross-linking reagents like mitomycin C but remains resistant to ionizing radiation. Considering that it is a partial deletion mutant, it is possible that this recR mutant maintains some degree of activity. The roles of the RecF pathway in *D. radiodurans*’s radioresistance still need to be addressed experimentally. Here, we

<table>
<thead>
<tr>
<th>Strains</th>
<th>Relevant marker</th>
<th>Reference or resource</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> DH5α</td>
<td>hsdR17 recA1 endA1 lacZΔM15</td>
<td>Invitrogen</td>
</tr>
<tr>
<td><em>D. radiodurans</em> R1</td>
<td>ATCC 13939</td>
<td></td>
</tr>
<tr>
<td>MOA</td>
<td>As R1 but recO::aadA</td>
<td>This study</td>
</tr>
<tr>
<td>MOA-D</td>
<td>As MOA recO::aadA (pDO recO&lt;sup&gt;Δ&lt;/sup&gt;)</td>
<td>This study</td>
</tr>
<tr>
<td>MOA-E</td>
<td>As MOA recO::aadA (pEO recO&lt;sup&gt;Δ&lt;/sup&gt;)</td>
<td>This study</td>
</tr>
<tr>
<td>TNK106</td>
<td>As R1 but recA::cam</td>
<td>29</td>
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Plasmids

| pMD18-T | TA cloning vector |
| pRADK | *E. coli-D. radiodurans* shuttle vector carrying *D. radiodurans* groEL promoter |
| pEO | pRADK derivative expressing *D. radiodurans* recO |
| pDO | pRADK derivative expressing *E. coli* recO |

TABLE 1. Bacterial strains and plasmids
have successfully constructed a recO null mutant and the mutant phenotypes have been characterized.

Construction of a *D. radiodurans* recO null mutant. The bacterial strains and plasmids used in this study are shown in Table 1, and the primers used in this study are shown in Table 2. Disruption of the *D. radiodurans* recO gene (*recODr*) was performed using a deletion replacement method as described previously (9). The construction strategy for recO::aadA is shown in Fig. 1A. The streptomycin resistance gene (*kat-aadA*) was obtained from the *pkat-aadA* plasmid (30), which was digested with HindIII and BamHI. A 543-bp DNA fragment corresponding to the immediate upstream portion of the *dr0819* (*recODr*) initial codon was amplified using primers MO1 and MO2 containing a BamHI restriction site, and a 629-bp DNA fragment corresponding to the immediate downstream region of the *dr0819* (*recODr*) stop codon was amplified using primers MO3 (containing a HindIII restriction site) and MO4. The PCR products were digested with BamHI or HindIII and then ligated to a *kat-aadA* cassette. The tripartite ligation products were amplified using MO1 and MO4, and the resulting fragments were transformed into *D. radiodurans* R1 as described previously (8). The recO::aadA mutant strain was selected on TGY (0.5% tryptone, 0.1% glucose, 0.3% yeast extract) agar containing 8 μg/ml streptomycin. Mutants were confirmed by PCR using primers MO5 and MO6 and sequenced. A 998-bp DNA fragment corresponding to intact *dr0819* was the only product produced when the wild-type strain R1 chromosomal DNA was used as the template. In contrast, an approximately 1,181-bp product was obtained using genomic DNA isolated from streptomycin-resistant colonies. The 998-bp DNA fragment corresponding to intact *dr0819* was not observed in streptomycin-resistant colonies, indicating that the strain was homozygous for the *aadA* gene, displacing recO in streptomycin-resistant colonies (Fig. 1B). The resulting recO null mutant was designated MOA.

Disruption of the recO gene of *D. radiodurans* results in a growth defect. The influence of recO disruption on the growth rate was evaluated. The wild-type strain R1, the recO mutant

**TABLE 2. Primers used in this study**

<table>
<thead>
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<th>Primer</th>
<th>Sequence (5′–3′)*</th>
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<tbody>
<tr>
<td>MO1...</td>
<td>MGATTTCAATCGGAATTTGTCTTACT</td>
</tr>
<tr>
<td>MO2...</td>
<td>CGCGGATCCGAGCTGACGTCATAAGAAGA</td>
</tr>
<tr>
<td>MO3...</td>
<td>CCAAGCTTACATTTGACAAAAGAATGG</td>
</tr>
<tr>
<td>MO4...</td>
<td>GCAAGCGGGGCGGAATCATCCGTTT</td>
</tr>
<tr>
<td>MO5...</td>
<td>GAAAGCGAGTCACAGCCCTC</td>
</tr>
<tr>
<td>MO6...</td>
<td>GGATTTGAGGTCTGCAGGTGA</td>
</tr>
<tr>
<td>DOF...</td>
<td>CACCATTATGCTCACCGCACCGCA</td>
</tr>
<tr>
<td>DOR...</td>
<td>GAGGGATCTGCGCGTGAAGAAGTCCAAAG</td>
</tr>
<tr>
<td>EOF...</td>
<td>ACTCATATGGAAGGCTGGCAGCGCGCAT</td>
</tr>
<tr>
<td>EOR...</td>
<td>TTCAGCGTAATGCAGGGTAAT</td>
</tr>
</tbody>
</table>

*a* Underlining indicates restriction sites.

![FIG. 1. recO mutant construction and verification by PCR. (A) Schematic of the construction of the recO deletion mutation. The map of recO in the *D. radiodurans* chromosome (top) and recO after disruption by its replacement with a streptomycin resistance cassette (bottom) are shown. (B) Ethidium bromide-stained agarose gel illustrating that the mutant carries a homozygous deletion of dr0819::aadA. Lane 1, R1; lane 2, MOA; lane 3, DNA marker.](http://jb.asm.org/Downloaded from)
MOA, and the recA mutant TNK106 (7) cells were grown overnight (with streptomycin for MOA and with chloramphenicol for TNK106). Next, 500-μl cultures were diluted into 100 ml TGY broth without antibiotics and shaken at 30°C. Cell growth was monitored using a spectrophotometer at 3-h intervals. As shown in Fig. 2, MOA exhibited a severe growth defect compared with the growth of its parent strain, R1; however, the growth rate of MOA was similar to that of TNK106, a recA mutant, indicating that recO is essential for cell viability, just as recA is.

Disruption of the recO gene of D. radiodurans sensitizes this strain to DNA-damaging agents. Since RecO plays an important role in DNA repair, the influence of a recO mutation on the sensitivity of D. radiodurans to UV and gamma radiation was investigated. Cells for each assay were grown in TGY broth with the appropriate antibiotics to exponential phase (optical density at 600 nm, 0.4 to 0.6). For gamma irradiation, cultures were diluted to an appropriate concentration in 1.5-ml tubes and irradiated on ice with different doses for 1 h. For UV exposure, appropriate cells were plated onto TGY agar. After the medium was completely absorbed, the plates were exposed to UV at the dose rate of 1.0 J/cm²/s. Irradiated and mock-irradiated cultures were diluted to appropriate concentrations and spread onto TGY agar plates without antibiotics. Colonies were counted after incubation at 30°C for 3 to 4 days. Disruption of recO resulted in a dramatic sensitivity to UV
and gamma radiation, which was of the same order of magnitude as that observed for the recA mutant (Fig. 3A and B). A dose of 50 J/m² of UV radiation or 200 Gy gamma radiation was sufficient to kill 90% of MOA cells, indicating that recO is essential for UV and gamma radiation resistance.

RecO<sub>Dr</sub> only partially restores MOA gamma radiation resistance. In an effort to identify the gene responsible for the sensitivity to DNA-damaging reagents as a result of the disruption of strain MOA, a complementation plasmid with a complete recO gene from <i>D. radiodurans</i> or <i>E. coli</i> (recO<sub>Ec</sub>) was introduced into the disruptant. The clones capable of restoring the disruptant’s ability to resist UV and gamma irradiation damage were screened.

The complete <i>D. radiodurans</i> recO coding sequence was amplified from the genomic DNA of wild-type strain R1 using primers DOF and DOR. The fragment was inserted into the pRADK plasmid, which was digested with the same enzymes. The newly constructed plasmid was designated pDO. The <i>E. coli</i> recO complementary plasmid pPEO was constructed like pDO was, except that the gene was amplified using primers EOF and EOR and that <i>E. coli</i> DH5<sub>x</sub> genomic DNA was used as the template. The resulting complementary plasmids were confirmed by sequencing. The plasmids were transformed into MOA, and the constructs were selected on TGY agar containing 8 µg/ml streptomycin and 3 µg/ml chloramphenicol. The resulting constructs were designated MOA-D (complementation with pDO) and MOA-E (complementation with pPEO).

The sensitivities of these strains to UV and gamma irradiation were then examined. As shown in Fig. 3C and D, the MOA-D strain was as resistant to UV and gamma irradiation as wild-type R1, a further confirmation that the sensitivity phenotype of MOA was caused by the disruption of recO but not by the polar effect of gene replacement, which might affect the expression of upstream and/or downstream genes. The pPEO construct fully restored MOA's UV resistance, while it only partially compensated the gamma radiation sensitivity phenotype of the mutant strain. Two possible explanations exist: (i) the expression of recO<sub>Ec</sub> did not occur in <i>D. radiodurans</i>, and (ii) RecO<sub>Dr</sub> has species-specific features. Since recO<sub>Dr</sub> can fully restore MOA’s UV resistance, we consider the first possibility unlikely. RecO<sub>Dr</sub> and RecO<sub>Ec</sub> show low homology at the sequence level (4), and RecO<sub>Dr</sub> possesses a Zn domain within the C terminus which is absent from RecO<sub>Ec</sub>. Biochemical studies revealed (21) that the Zn domain of RecO<sub>Dr</sub> modulates its DNA binding activity. Furthermore, gamma radiation generates more types of DNA lesions than UV radiation (4). We hypothesize that some DNA lesions, those only generated in response to ionizing radiation, may require the species-specific features of recO<sub>Ec</sub>. However, further experiments are needed to confirm the species-specific features of RecO<sub>Dr</sub>.

Here we reported the first evidence of the vital role of recO in <i>D. radiodurans</i>’s radiosensitivity. A <i>D. radiodurans</i> recO null mutant was constructed, and it demonstrated recA mutant-like phenotypes: slow growth and extreme sensitivity to gamma and UV radiation. This work provides useful insight into the DNA repair mechanisms exhibited by <i>D. radiodurans</i>.

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REFERENCES


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