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LexA Analog (dra0074) Is a Regulatory Protein That Is Irrelevant to *recA* Induction

Duohong Sheng[1], Zhiguo Zheng[1], Bing Tian[1], Binghui Shen[2] and Yuejin Hua[1,*]

[1]Institute of Nuclear-Agricultural Sciences, Zhejiang University, 310029, Hangzhou, China; and [2]Department of Radiation Biology, City of Hope National Medical Center and Beckman Research Institute, Duarte, CA 91010-3000, USA

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The protein DRA0074 is suggested to be another LexA in *Deinococcus radiodurans*, having similar motifs and RecA-mediated cleavage activity to *D. radiodurans* LexA (dra0344). However, its function has not been studied. We disrupted the gene dra0074 and measured its effect on RecA induction using fusion translation, immunoblot, and proteomic analysis. Results showed that the product of gene dra0074 is not involved in RecA induction, but is a regulator of other metabolisms in *D. radiodurans*.

Key words: *Deinococcus radiodurans*, LexA, *recA* induction.

The polyextremophile bacterium *Deinococcus radiodurans* is most famous for its remarkable resistance to severe damage caused by ionizing radiation (IR), desiccation, UV radiation, and oxidizing agents (*1*, *2*). This resistance is known to be due to extremely proficient DNA repair processes in *D. radiodurans*, and RecA plays a critical role in the process (*3*, *4*). Disruption of *recA* results in a remarkable reduction in its ability to recover from acute DNA damage (*5*, *6*). In *E. coli*, RecA was thought to be regulated by several gene products, including RecA, LexA, and DinI. LexA acts as a repressor in RecA regulation (*8–11*). However, Narumi *et al.* demonstrated that *D. radiodurans* LexA protein (dra0344) is not involved in RecA induction following γ irradiation (*13*, *14*). It is intriguing to find the regulatory mechanism of RecA in the extremely radio-resistant bacterium, *D. radiodurans*.

Gene dra0074 was suggested to encode another LexA analog in *D. radiodurans* (*12*, *14*). Multiple amino acid sequence alignment showed that the protein (dra0074) has similar motifs to *D. radiodurans* LexA (dra0344) and *E. coli* LexA (Fig. 1A). The N-terminal domain involved in DNA binding forms a variant helix-turn-helix DNA-binding motif, and the C-terminal domain involved in the cleavage reaction is also conserved in the product of gene dra0074 (Ala-130, Gly-131, Ser-163, and Lys-203). Therefore, determining whether this protein acts as a repressor in RecA induction is important for understanding the regulation of RecA in the highly radio-resistance bacterium.

We constructed a null mutant of gene dra0074 to study its function. Transcriptional and immunoblot assays showed that gene dra0074 is not involved in RecA induction. Two-dimensional (2-D) gel electrophoresis confirmed that the gene is irrelevant to the DNA repair induced by ionizing radiation (IR), but is involved in other metabolic process in *D. radiodurans*.

*To whom correspondence should be addressed. E-mail: yjhua@zju.edu.cn

MATERIALS AND METHODS

Strains, Plasmids and Growth Conditions—*D. radiodurans* strain R1 used in the study was grown in TGY broth (0.8% Bacto Tryptone, 0.1% Glucose, 0.4% Bacto Yeast Extract) at 32°C with aeration or on TGY plate solidified with 1.7% agar. *E. coli* TG1 was purchased from Invitrogen (La Jolla, Canada) and grown in Luria-Bertani broth at 37°C with aeration, or on Luria-Bertani plates containing 1.7% agar. A modified shuttle plasmid pRADZ3 (*15*), a gift from M.E. Lidstrom of the University of Washington, carried a kanamycin resistance gene in place of the chloramphenicol resistance gene on it. Subclone plasmid pGEM®–T Easy vector was purchased from Promega Co. Antibiotics were added in the medium when necessary. For *E. coli*, the medium was supplemented with 100 $\mu g \cdot ml^{-1}$ of ampicillin or 30 $\mu g \cdot ml^{-1}$ of kanamycin. For *D. radiodurans*, 4 $\mu g \cdot ml^{-1}$ of kanamycin or 3 $\mu g \cdot ml^{-1}$ of chloramphenicol was added to the medium.

General DNA Manipulations—Plasmids and chromosomal DNA were isolated by the alkaline lysis method from *E. coli* or by lysozyme lysis method from *D. radiodurans* (*16*, *17*). The cloning of gene dra0074 was performed according to general procedures. The gene was amplified by PCR with the primers 5′ CGCATATGCGAACATTTCAGTACG 3′ and 5′ AAGGATCCAGGCGGTGGTGAGATT 3′, then ligated into pGEM®–T Easy Vector. The recombination plasmid was transformed into TG1 by $CaCl_2$ method (*16*).

Gene dra0074 was disrupted using the direct insertional mutagenesis technique described by T. Funayama *et al.* (*18*) with modifications (Fig. 2A). The recombination plasmid with the gene dra0074 was pretreated with *Nde*I to remove a *Hin*cII sites on the plasmid, then digested with *Hin*cII and ligated to a 914 bps *Hin*cII fragment containing the chloramphenicol-resistance gene (*cat*) from pKatCAT. The plasmid was linearized with *Ssp*I and transfected into *D. radiodurans* R1 (*19*, *20*). The mutant M74 was screened out on TGY agar with chloramphenicol.

To analyze the transcription of RecA in *D. radiodurans*, *recA-lacZ* transcriptional fusion was constructed. PCR product containing the full length of *recA*

A

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E coli LexA          1                                MKA----LTARQQEVFD-LIRDHIS 20
S coelicolor LexA    1            MSDAANPEGHKRSLPGRPPGIRADSSGLTDRQRRVIE-VIRDSVQ 44
dra0344              1                               MTRRAAN--CAHMPPELTP-TRRSILQ 24
dra0074              1   MRTFQYANANPSANVSPMGRTSLATSAAKARGFLNGLIAERMRQLNLSTVKDFAD 55
                                                H1          H2          H3
E coli LexA         21   ---QTGMPPTRA-EIAQRLGFRSPNAAEEHLKALARKGVIE---------IVSGA 62
S coelicolor LexA   45   ---RRGVPPSMR-EIGQAVGLSSTSSVAHQLMALERKGFLRRDPHRPRAYEVRGS 95
dra0344             25   ATLRLGAGATAG-QVAQEVGIT-KQAISQQVNILRKLGYLQP------AETRYGP 71
dra0074             56   ---YAGIGRTSIHDLVRGRTTTSGTWTKPSFDTLTKLAVALDKPTHELLYLIDPE 107
                                                H4
E coli LexA         63   SRGIRLLQEEE-EG------LPLVGRVAAGEPLLAQQHIEGHYQVDPSLFKPNA- 109
S coelicolor LexA   96   DQAASVQPTDT-AGKPAASYVPLVGRIAAGGPILAEESVEDVFPLPRQLVGDGE- 148
dra0344             72   LQVTDRARAALGEG------LPIYGQIAAGIPALAEQSPEDFTPSIEALLGLKAG 120
dra0074            108   APGANLMFDVQQVF------VYIAGQVGAG-PQQLUES-SDVVYVERQFAENRD- 153

E coli LexA         110   DFLLRVSGMSMKD--IGIMDGDLLAV-HKTQDVRNGQVVVARI--DDEVTVKRLK 159
S coelicolor LexA   149   LFVLKVVGDSMIE--AAICDGDWVIV-RRQPVAENGDIVAAML--DGEATVKRFK 198
dra0344             121   DFLLRVRGESMTG--IGVMDGDYVVV-RPAPEVHDGEVAVVLVPGDNAATLKRLY 172
dra0074             154   LIAFTVVGDSMAGGRHPIHDGDVVIVDQRVGGEVNFPVVARLK--DDGHVVKRCG 206

E coli LexA         160   KQGNKVELLPENSEFKPIVVDLRQQSFTIEGLAVGVIRNGDWL        202
S coelicolor LexA   199   REDGHVWLLPHNSAYEPIPGDD----ATILGKVVAVLRRV          234
dra0344             173   HFGQDILLTSENPAMPRLSFPAEQ--VQVQGRMVGRVGVGAPRVSHRVTE 220
dra0074             207   PAASSTAFTRTSWTLTPQSLPL-----TV-SPTWWDASSG           240
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B

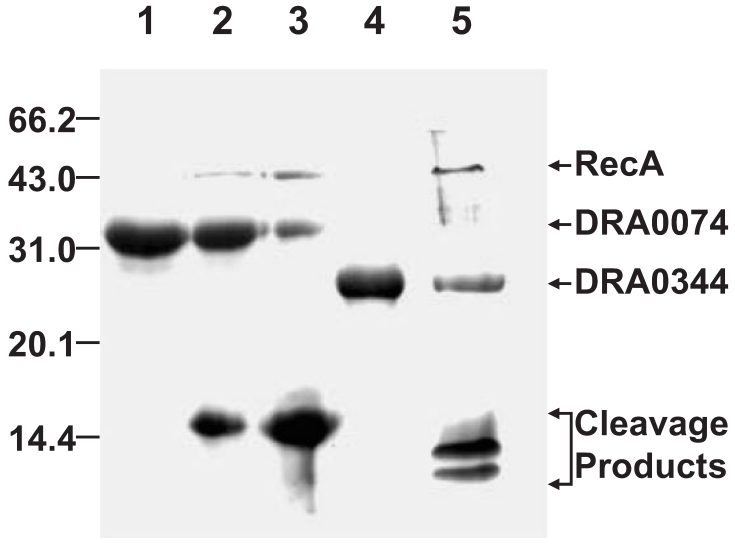

Fig. 1. **Alignment of the amino acid sequences and RecA-mediated cleavage assay.** (A) Multiple amino acid sequence alignment of *E. coli* LexA, *D. radiodurans* LexA dra0344 and dra0074. The identical residues are marked in red. The conserved alanine and glycine residues in the cleavage site and the serine and lysine residues required for cleavage are indicated by filled triangles. The locations of four α helices (H1, H2, H3, and H4) found in gene dra0074 are underlined in the sequence. (B) RecA-mediated cleavage assay. *D. radiodurans* LexA (dra0074) was tested at 30 μM and LexA (dra0344) was used as a control. Lane 1, LexA (dra0074) only; Lane 2, LexA (dra0074) with RecA (1 μM); Lane 3, LexA (dra0074) with 3 μM RecA; Lane 4, LexA (dra0344) only; lane 5, LexA (dra0344) with 3 μM RecA.

promoter (about 2,071 bps) was obtained with the primers of RecA-F (5′ GTCTAGAGAAACACCAGCATGATCGC 3′) and RecA-R (5′ GGGCCCTTGCTCATGGGTGCTCCT 3′), and subsequently cloned between *Bgl*II and *Spe*I sites on the modified pRADZ3 (*15*). The resulting plasmids were transformed into *D. radiodurans* by a double crossover with selection for kanamycin resistance.

Growth and Resistance to IR—D. radiodurans strains were grown on TGY agar, and the colonies were scored every 12 h to generate growth curves. Only *D. radiodurans* cultures in the early stationary phase were evaluated for their ability to survive ionizing radiation. Briefly, cell suspension (1 ml) was irradiated at room temperature for 1 h with ^{60}Co γ-rays at different doses (from 2 to 8 kGy), which were adjusted by changing the distance of samples from the γ-ray source. Irradiated cultures were diluted, plated in triplicate on TGY agar plates, and incubated for 3 days at 30°C before scoring for survivors. Survival was determined by counting colony-forming units (CFU).

LexA Cleavage Assay—LexA cleavage was examined by a modified procedure of K. Satoh (*21*). The reaction contained 25 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 6 mM oligonucleotide, 1 mM ATP, 30 μM *D. radiodurans* LexA (dra0074), and gradient RecA. First, the mix without LexA was preincubated at 37°C for 10 min. Then, the reaction was initiated by the addition of LexA. After incubation at 37°C for 1.5 hours, the reaction was quenched, and subse-

quently analyzed by 15% SDS–polyacrylamide gel. LexA cleavage products were visualized by silver staining.

β-Galactosidase Assay and Western Blot—Expression of the *lacZ* reporter gene in *E. coli* and *D. radiodurans* colonies was detected using X-Gal (40 μg/ml). β-galactosidase activity in *D. radiodurans* was measured in toluene-permeabilized cells by the method of R. Meima (*22*).

Cells were disrupted with an ultrasonicator at 600 W output for a total of 5 min on ice and the debris was removed by centrifugation (16,000 × *g*, 20min). Supernatant protein was determined using the Bradford protein dye assay and used for western analysis following the standard protocol (*17*).

Two-Dimensional (2-D) Gel Electrophoresis—2-D gel electrophoresis was performed according to the manufacturer's instruction (Amersham Biosciences, Uppsala, Sweden). Briefly, Immobiline DryStrip gels (pH 4–7, 24 cm) were rehydrated in 24 cm strip holders and 200 μg of protein was loaded using in-gel rehydration, then electrofocused until the total volt-hours reached 64.0 kVh. After isoelectric focusing, the strips were equilibrated twice with gentle shaking for 15 min in SDS equilibration buffer. The first step was performed in an equilibration solution containing 6 M urea, 30% w/v glycerol, 2% w/v SDS, 1% w/v DTT, 50 mM Tris-HCl buffer (pH 8.8) and 0.002% w/v bromophenol blue. The second step was performed in the equilibration solution as above, but DTT was replaced by 2.5% w/v iodoacetamide. The second dimension was run with Ettan DALTsix electrophoresis Unit (Amersham Biosciences) at 1500 mW.

Silver staining was according to F. Gharahdaghi *et al.* (*23*). Stained gels were scanned with ImageScanner (Amersham Biosciences) and analyzed using ImageMaster 2D Elite software supplied by the manufacturer.

MALDI-TOF Mass Spectrometric (MS) Analysis and Database Search—Chemical destaining and enzymatic digestion of protein spots were performed as previously described (*23–25*). Mass spectrometric analysis was carried out using the protocol developed by Gharahdaghi *et al.* (*26*) with some modifications. Peptide mixtures 1 μl were mixed with an equal volume of 10 mg/ml a-cyano-4-hydroxycinnamic acid (Sigma) saturated with 50% ACN in 0.05% TFA. The sample was then analyzed with a Voyager-DE STR MALDI-TOF MS using a delayed ion extraction and ion mirror reflector mass spectrometer (Applied Biosystems, Foster City, CA, USA). The instrument setting was reflector mode with 160 ns delay extraction time, positive polarity, 60–65% grid voltage, and 20,000 V accelerating voltage. Laser shots at 200 per spectrum were used to acquire the spectra with mass range from 1,000–4,000 Da. External calibration was carried out using a Peptide Mass Standard Kit (Perspective Biosystems). The matrix and the autolytic peaks of trypsin served as internal standards for mass calibration.

The mass spectra were interpreted with the Mascot peptide mass fingerprint program available on the web site (http://www.matrixscience.com/).

RESULTS

RecA Can Promote the Proteolytic Activity of LexA—In Fig. 1A, alignment of the amino acid sequences revealed that dra0074 has a typical LexA domain, and its amino acid residues involved in cleavage are also conserved. RecA-mediated cleavage activity of the protein was investigated (Fig. 1B). The expression, purification and refolding of the insoluble dra0074 protein will be reported elsewhere. With *D. radiodurans* RecA, the cleavage products from dra0074 protein appeared in lane 3 and lane 4. This result is in agreement with previous suggestion that dra0074 is another LexA in *D. radiodurans* (*12*, *14*).

In the present work, we found that it was difficult to express the soluble dra0074 protein in *E. coli*. Attempts

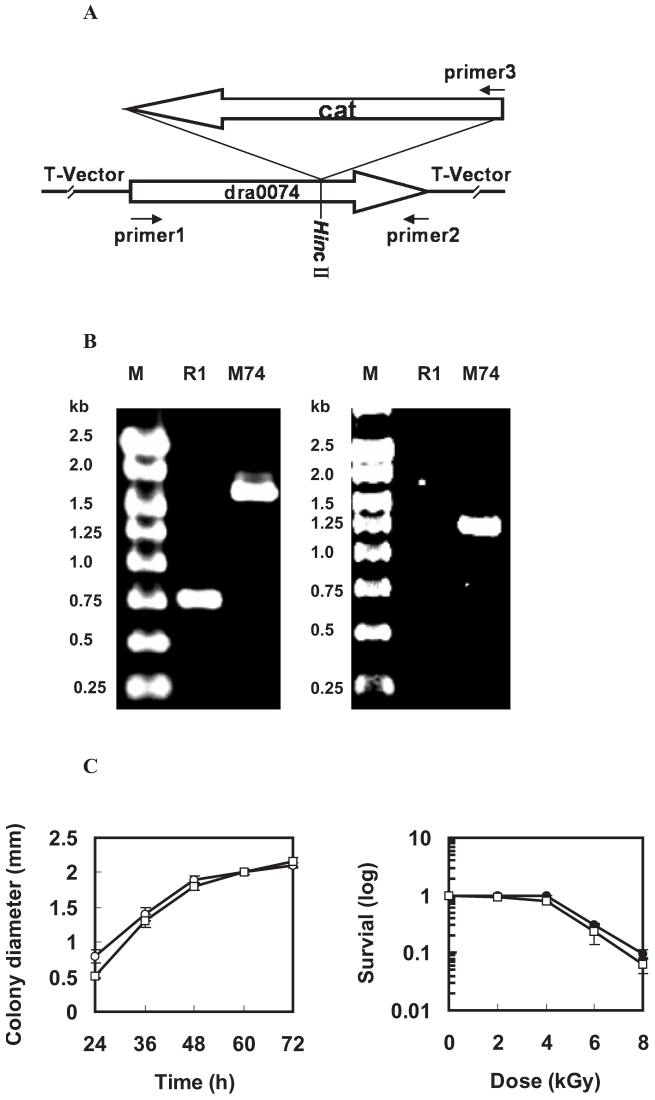

Fig. 2. **Mutant analysis.** (A) Construction of the mutant. The arrows indicate the predicted direction of transcription. Primers used for PCR analysis are designated with thin arrows at their relative positions: primer 1, 5′ CGCATATGCGAACATTTCAGTACG 3′; primer 2, 5′ AAGGATCCAGGCGGTG GTGAGATT 3′; primer 3, 5′ AGAGCCAGCGAGGCGAAGGAGA 3′. (B) The mutant was confirmed by PCR. R1 and M74 were inspected by PCR and electrophoresed in agarose gel. Primer 1 and primer 2 were used in the left map to test the insertion, and primer 1 and primer 3 were used in the right map to confirm the predicted direction of transcription. (C) The growth curves and representative survival curves for *D. radiodurans* mutant strain M74 (open squares) and wild type strain R1 (closed circles). Values are the means ± standard deviations obtained from four independent experiments.

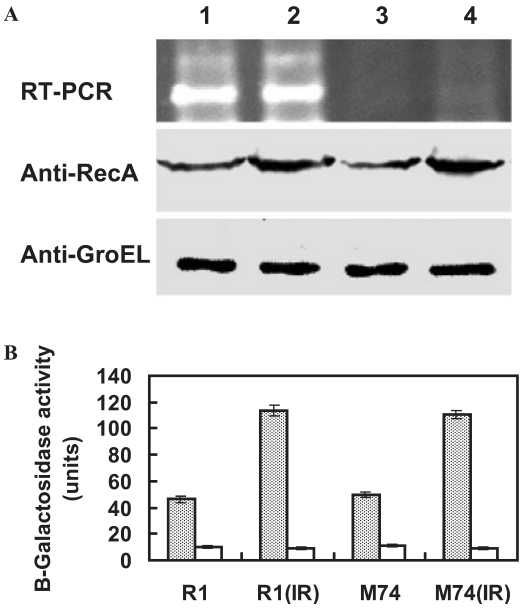

Fig. 3. **Western blot and transcriptional activity assay.** (A) Western blot. 40 μg of protein was loaded on each lane of the SDS-gels. Immunoblot analyses were carried out with the anti-RecA antibody. A total of 2 μg of RNA was used for RT-PCR following the general protocol, and the results are shown in the upper panel as a control. Lane 1, R1 without irradiation; Lane 2, R1 irradiated at 2 kGy; Lane 3, M74 without irradiation; Lane 4, M74 irradiated at 2 kGy. Anti-GroEL bands were used for sample loading control. (B) Transcriptional activity assay. Lane1, R1 without irradiation; Lane 2, R1 irradiated at 2 kGy; Lane 3, M74 without irradiation; Lane 4, M74 irradiated at 2 kGy. β-Galactosidase activity was determined using a modification of the method of Guarante (1983). As a control, the plasmid without the *lacZ* gene (shown with white bars) was assayed for endogenous β-galactosidase activity in cultured cells. Values are the means ± standard deviations obtained from four independent experiments.

resulted in the death of the host cell, indicating that the protein plays an important role *in vivo*.

Disruption of Gene dra0074—Mutant strain M74 was verified by PCR with the primers marked in Fig. 2A. In strain R1, the amplifying band was about 723 bps, in accordance with the normal gene. However, a band of 1637 bps, total size of the gene dra0074 and the inserted fragment, appeared in the mutant (left in Fig. 2B). In addition, PCR with primer 1 and primer 3 showed that the PCR product about 1477 bps only appeared in the mutant (right in Fig. 2B), indicating that the *cat* fragment was inserted in the reverse orientation in gene dra0074.

The mutation was further confirmed by RT-PCR as described by Sambrook *et al.* (*16*). Figure 3A shows that gene dra0074 was not amplified in the mutant M74, suggesting that the gene was disrupted by the inserted *cat* fragment.

In *E. coli*, inactivation of LexA leads to lethal filament (*13*). In *D. radiodurans*, however, disruption of gene dra0074 has no significant effect on the growth curves (Fig. 2C), but M74 has a longer delay period. The resistance curves of the mutant are also shown in Fig. 2C. The absence of gene dra0074 repressed on the survival of *D. radiodurans*, but not significantly. Based on these results, we suggest that *D. radiodurans* gene dra0074 might play a different role from that of LexA in *E. coli*.

Absence of Gene dra0074 Does Not Affect RecA Induction—To study the effect of gene dra0074 on RecA induction in *D. radiodurans*, expression of RecA was examined by Western Blot and transcriptional activity assay. After irradiation at 2kGy, RecA protein was detected with the anti-RecA antibodies (rabbit IgG, laboratory stock). Expression of gene dr0074 was examined as a control by RT-PCR with the primers of gene dra0074 (Fig. 3A). The absence of gene dra0074 did not alter RecA quantities in the cells. After irradiation, RecA exhibited a similar induction in wild type R1 as in mutant M74. In strain R1, RecA increased about 2 fold after irradiation, and the expression of gene dra0074 changed very little at the same time. In strain M74, RecA exhibited a similar increase (about 2 fold after irradiation) in the absence of gene dra0074.

Transcriptional analysis of *recA* promoter in R1 and M74 was carried out (Fig. 3B). In strain R1, the β-galactosidase activity was about 46 units, and 113 units after the IR treatment. In strain M74, it was 50 units, and 110 units after induction. The β-galactosidase activities show little difference between R1 and M74, indicating that gene dra0074 is not involved in RecA induction, corresponding with the results from immunoblotting.

Gene dra0074 Is Not Involved in the Radiation-Induced DNA Damage Response, but Some Metabolism in D. radiodurans—To verify the function of gene dra0074 in *D. radiodurans*, we compared proteomic patterns between wild type R1 and mutant M74 (Fig. 4). In the map of R1, without irradiation, 466 well-resolved proteins were detected in the pH range from 4 to 7 after silver staining, However, 401 proteins were detected in the map of M74. After the irradiation, 436 and 415 proteins were resolved in the maps of R1 (IR) and M74 (IR) respectively, and expression of many proteins in the two strains was altered obviously. Differences were found in the 2-D maps between R1 and M74. In spite of the irradiation, many spots decreased in intensity and only a few spots increased in intensity in strain M74. Gene dra0074 seemed to be related to constitutive expression of some proteins. In addition, the absence of gene dra0074 did not affect these IR induced proteins. After irradiation at 2 kGy, the mutant M74 exhibited similar protein inductivity to the wild type R1 (Fig. 4, B and D). Based on the comparison, we suggest that the product of dra0074 might act as a regulator of normal expression of genes that are not irrelated to DNA damage response caused by irradiation. To prove this hypothesis, 26 protein spots that were induced by the irradiation or clearly altered by mutation were excised from gels and subjected to peptide mass fingerprinting analysis to assign putative functions to the proteins.

Ten of 12 radiation-induced spots were successfully identified by MALDI-TOF mass spectra measurement and database search (Table 1). These spots included some key proteins in IR resistance of *D. radiodurans*, such as SSB, PprA, RecA (*5*, *7*, *27*, *28*). Most of the them showed similar induction in M74 and R1, but spots 10 and 11 that were down regulated in M74 after irradiation. Spots 10 and 11 were identified as a proline iminopeptidase-related protein and a peptidase-related protein, which

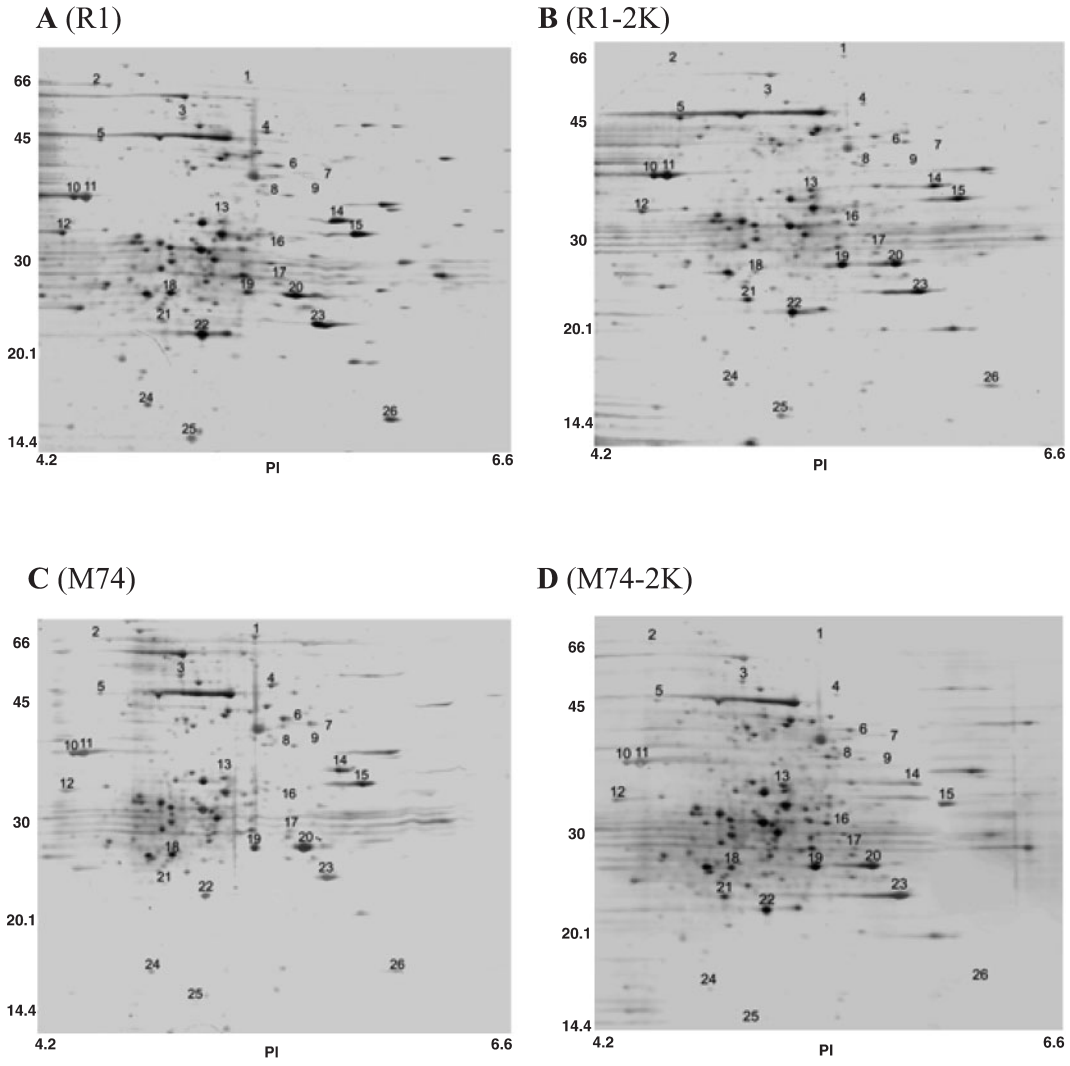

Fig. 4. **2-D PAGE analysis of cellular proteins of *D. radiodurans*.** Extracted proteins were separated by isoelectric focusing in the pH range from 4 to 7 in the first dimension and 12.5% vertical SDS-PAGE in the second dimension. Resolved proteins were visualized following silver staining. (A) R1, unirradiated; (B) R1, irradiated at 2 kGy; (C) M74, unirradiated; (D) M74, irradiated at 2 kGy. Analyzed spots were numbered, and the numbers match in the four maps.

are belonging to hydrolase involved in protein degradation. Therefore, gene dra0074 was not found to participate in the DNA damage response induced by radiation.

To elucidate the function of gene dra0074, 14 proteins whose expression were obviously altered in M74 were identified and shown in Table 2. Their functions include carbohydrate transport and metabolism, lipid transport and metabolism, inorganic ion transport and metabolism, transcription and translation, protein metabolism and chaperone. None of the identified proteins directly participated in DNA damage repair. We suggested that gene dra0074 is involved in one or more metabolic pathways other than DNA damage response caused by irradiation.

DISCUSSION

This study is part of an effort to understand the mechanism of RecA regulation in *D. radiodurans*. In most eubacteria, RecA induced expression is repressed by LexA, a key protein in the inducible DNA repair system (the SOS system). However, Narumi *et al.* have demonstrated that the *D. radiodurans* LexA protein (dra0344) is not involved in RecA induction following γ-irradiation (*13*, *14*). In this study, we revealed that another *D. radiodurans* LexA (dra0074), which has similar motifs to *D. radiodurans* LexA (dra0344), LexAs from *E. coli* and *S. coelicolor,* is also irrelevant to *recA* expression, although it does exhibit RecA-mediated cleavage activity. An attempt to construct a double mutant of dra0074 and dra0344 failed, perhaps because these two proteins have similar regulatory functions in key bacterial metabolic pathways. Nevertheless, our result sheds light on the RecA regulation in *D. radiodurans*.

D. radiodurans survives more than 100 DNA DSBs after exposure to 10 kGy of γ-radiation. Hyper-radioresistance of *D. radiodurans* is not only based on its unique genomic structure (*29*) but also on its efficient RecA-dependent DSB homologous recombinational repair and DNA damage responses (*30*). Although the regulation of RecA in *D. radiodurans* is still unresolved,

Table 1. **Protein spots induced following γ-radiation. MALDI-TOF mass spectra measurement and database searching identified these spots.**

Spot number	Identified protein	Gene number	R1	R1 (IR)	M74	M74 (IR)
1	Acyl-CoA dehydrogenase, putative	DR2361	+	++	++	++
6	Critrate synthase	DR0757	+	++	+	++
7	Acetyl-CoA acetyltranceferase	DR2480	+	++	+	++
9	RecA protein	DR2340	+	+++	+	+++
10	Proline iminopeptidase-related protein	DR0654	+	+++	+	–
11	Peptidase-related protein	DR0478	+	+++	+	–
13	Single-strand binding protein (SSB)	DR0099	+	++++	+	++++
16	DNA damage repair protein (PprA)	DRA0346	+	+++++	+	+++++
17	Enoyl-acyl carrier protein reductase	DR1967	+	++	++	+++
19	Unknown		+	+++	++	+++
21	Unknown		+	+++++++	+	+++++++
25	Tellurium resistance protein TerB putative	DR2220	+	+++	+	+++

The changes of the protein expression in the maps were evaluated among R1, M74, R1 (IR), and M74 (IR). The latter are marked (IR), meaning irradiation at 2 kGy. The plus sign represents an induced protein, and the number following the plus sign represents the intensity of the protein. The minus sign represents a repressed protein in the same way. Protein PprA has not been reported formally. Identified proteins scores in MASCOT database are all significant ($p < 0.05$)

Table 2. **Protein spots whose expression levels altered in the mutant.**

Spot number	Identified protein	Gene number	MW	PI	Coverage (%)
Inorganic ion transport and metabolism					
8	Potassium channel, beta subunit, putative	DR2317	35,134	5.33	54
23	Superoxide dismutase (sodA), Mn family	DR1279	23,464	5.55	34
Carbohydrate transport and metabolism					
3	Aconitate hydratase, B subunit	DR1720	97,941	4.94	15
14	Succinyl-CoA synthetase, alpha subunit	DR1248	30,864	5.47	33
15	Fructose-bisphosphate aldolase	DR1589	32,401	5.48	69
18	Phosphoglycerate mutase	DR0278	25,102	5.348	45
Lipid transport and metabolism					
20	Phage shock protein A	DR1473	24,591	5.51	51
Transcription and translation					
22	Elongation factor P	DR0119	20,462	5.10	45
24	Transcription elongation factor	DR1970	18,053	4.94	55
Protein metabolism and chaperone					
2	Serine protease, subtilase family	DRA0283	76,708	4.90	29
4	GroEL protein	DR0607	57,742	5.03	43
10	Proline iminopeptidase-related protein	DR0654	34,906	4.46	46
11	Peptidase-related protein	DR0478	57,742	5.03	46
Unknown spots					
26	Hypothetical protein	DR1768	15,164	7.93	35

MALDI-TOF mass spectra measurement and database searching identified these spots. Classification of proteins functions is based on NCBI's clusters of orthologous groups (COGs). Identified proteins scores in MASCOT database are all significant ($p < 0.05$).

PprI (or IrrE) was suggested to play a critical role in RecA induction. Disruption of *pprI* inhibited *recA* expression markedly, and the mutant lost most of the IR resistance, indicating that it is a regulator located upstream of the RecA protein induction pathway (*27*, *31*). In addition, B.D. Almeida *et al.* (2002) thought RecA was another protein involved in *recA* expression. Cells bearing the *recA*670 mutation showed an elevated constitutive *recA* expression in the absence of DNA damage (*13*). Recently, we observed that RecX could repress *recA* induction and revealed a negative regulation mechanism on *D. radiodurans recA* expression (data will be published elsewhere). Based on the above results, *D. radiodurans* exhibited an unusual mechanism underlying the regulation of RecA (*13*, *14*, *27*).

As a core technology of proteomics, two-dimensional electrophoresis combined with mass spectrometry is the most common methodology for assessing large-scale protein expression profile changes (*24*, *26*). We compared the radiation-induced protein pattern of wild type with that of the mutant. We demonstrate that gene dra0074 was irrelevant to DNA damage response caused by irradiation, but relevant to other metabolisms in *D. radiodurans*. It might act as a regulator in some other metabolic pathway to help recover from extreme adverse physiological conditions such as starvation. This corresponds to White's finding that proteins encoded on chromosome II are involved in energy metabolism and may play roles in the recovery of *D. radiodurans* from prolonged periods of desiccation or starvation (*9*). In the

present work, only a small number of protein spots whose expression was altered in the mutant were identified, but they were representative of the mutant and sufficient to back up our hypothesis. The remaining protein spots will be identified subsequently to explain in detail the role of gene dra0074 in *D. radiodurans*.

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