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Analysis of a factor released from UV light-irradiated *Dictyostelium discoideum*

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SUMMARY

The behavior of the social amoeba *Dictyostelium discoideum* following UV irradiation was analyzed. Cells cultured in a liquid medium migrated at 4.5 ± 0.7 μm/min, while their motility increased to 19.4 ± 1.7 μm/min following UV irradiation (50 J/m^2). A conditioned medium prepared from the irradiated cells, CM (UV), was used in the following experiments. Motility of cells that had not been exposed to UV increased to 29.7 ± 2.8 μm/min when the medium was changed to CM (UV). In addition, the expression levels of *recA* and *rad51* were increased and sensitivity to UV was decreased in these cells. These results indicate that UV light-irradiated *D. discoideum* cells release a factor that enhances motility and induces expression of DNA repair enzymes in unirradiated cells. The factor would be important for *D. discoideum* to alleviate damage.

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INTRODUCTION

It is important for organisms to maintain their genomic integrity. However, they are continuously exposed to various DNA-damaging agents such as UV light, ionizing radiation and chemicals. Organisms therefore have mechanisms by which DNA damage is identified and repaired.

The social amoeba *Dictyostelium discoideum*, a lower eukaryote feeding on bacteria, has been shown to be resistant to DNA-damaging agents (Deering, 1988; Yu et al., 1998). Several genes responsible for DNA repair have been found and characterized in this organism. APNA, formerly called DdAPN, and APEA are AP endonucleases (Freeland et al., 1996; Tsuji et al., 2001). *repB*, *repD* and *repE* encode the homologs of the human XPB DNA helicase, XPD DNA helicase and the damage recognition molecule UVDDB/XPE, respectively (Alexander et al., 1996; Lee et al., 1997). A Rad51 homolog has been found in the database (http://www.csm.biol.tsukuba.ac.jp/cDNAproject.html). Recently, a RecA homolog localizing in mitochondria has been identified and characterized (Hasegawa et al., 2004). Lesions in the *D. discoideum* genome are repaired by these enzymes.

It is also important for organisms to take refuge when they are exposed to DNA-damaging agents. If there are agents nearby the organisms, the organisms should escape from them as soon as possible to avoid lethal damage. Experimental result demonstrating that *D. discoideum* takes refuge when exposed to UV light is shown in this report. In addition, the experimental evidence showing here demonstrates that UV light-irradiated *D. discoideum* released a factor that increased motility and induced DNA repair enzymes in unirradiated cells.

MATERIALS AND METHODS

Cell line and culture condition

D. discoideum Ax2 was used in the experiments. Cells were cultured in HL5 medium (0.04% KH_2PO_4, 0.05% Na_2HPO_4, 0.7% yeast extract, 1.4% bacteriological peptone and 1% glucose, pH 6.5).

Motility assay of UV light-irradiated cells

D. discoideum cells were placed with 20 µl HL5 medium on a glass slide at a density of 2.0×10^4 cells/cm^2. After 20 min, the medium was removed from the glass slide. The cells on the slide were irradiated by 50-J/m^2 UV light (254 nm) by the use of a UV crosslinker CL-1000 (UVP, CA, USA) and then incubated with a fresh HL5 medium (20 µl). Cells on the slide were photographed at 2-min intervals (~20 min) to determine migration speed. For the control, cells were placed with 20 µl HL5 medium on a slide at a density of 2.0×10^4 cells/cm^2. After 20 min, the medium was changed to 20 µl fresh HL5 medium. These cells were also photographed at 2-min intervals to determine the speed. Some of the cells did not migrate either in control or exposed fractions. Cell motility was determined without these cells.

Motility assay in conditioned medium

Preparation of conditioned medium: Cells were placed with 20 µl HL5 medium on a glass slide at a density of 2.0×10^4 cells/cm^2 for 20 min. The medium was changed to 20 µl fresh HL5 medium. After 20 min, the medium was removed from the slide and used as a control conditioned medium, CM. Preparation of conditioned medium of UV light-irradiated cells: Cells were placed with 20 µl HL5 medium on a glass slide at a density of 2.0×10^4 cells/cm^2. After 20 min, the medium was removed from the slide. The cells on the slide were irradiated by 50-J/m^2 UV light and then incubated with 20 µl fresh HL5 medium for 20 min. The medium in which UV light-irradiated cells were incubated, CM(UV), was recovered. Assays: Cells were placed with HL5 medium on two slides (2.0×10^4 cells/cm^2). HL5 medium on the slides was replaced by CM or CM(UV). Cells were photographed at 2-min intervals (~20 min) to determine migration speed.

Gene expression analysis

Preparation of conditioned medium: Cells were cultured with 10 ml HL5 medium in a 90-mm dish until the density reached 6.0×10^4 cells/cm^2. Medium in the dish was removed, and the cells were rinsed with a phosphate buffer (1.66 g NaH_2PO_4 in 1 L H_2O, pH adjusted to 6.1 with KOH). Cells were then incubated with 10 ml fresh HL5 medium for 20 min. The medium was removed from the dish and used as a control conditioned medium, CM. Preparation of conditioned medium of UV light-irradiated cells: Cells were cultured with 10 ml HL5 medium in a 90-mm dish until the density reached 6.0×10^4 cells/cm^2. The cells in the dish were rinsed with the phosphate buffer, irradiated by 50-J/m^2 UV light, and then incubated with 10 ml fresh HL5 medium for 20 min. The medium in which UV light-irradiated cells were cultured, CM(UV), was recovered. Assays: Cells were cultured with 10 ml HL5 medium in two

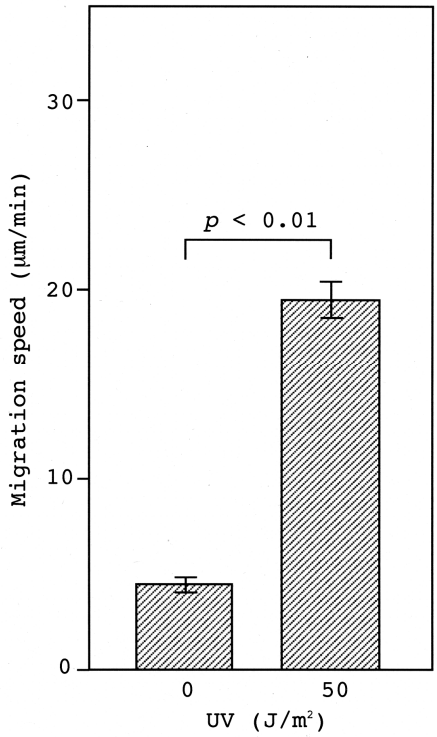

Fig. 1. Motility of cells exposed to UV light. Unexposed cells (control cells) and UV light-irradiated cells were photographed at 2-min intervals to determine migration speed. Migration speed (mean ± S.E.) was determined from 29 cells (control cells) and 41 cells (UV light-irradiated cells) obtained from two independent experiments.

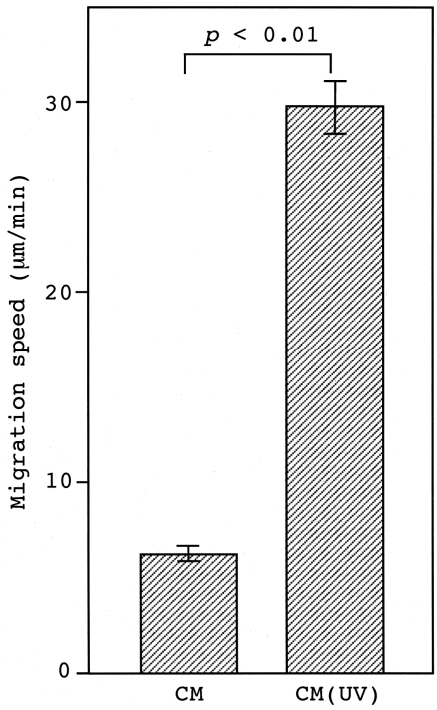

Fig. 2. Motility of cells in the conditioned medium. Cell migration speed in CM and CM(UV) was analyzed. Migration speed (mean ± S.E.) was measured from 29 cells in CM and 21 cells in CM (UV) obtained from two independent experiments.

dishes (6.0 x 10^4 cells/cm^2). HL5 medium in the dishes was replaced by CM or CM(UV). After 40 min, cells were harvested for RNA preparation. Expression levels of *recA* and *rad51* were analyzed by RT-PCR by the use of a One Step RT-PCR Kit (Takara Shuzo, Kyoto, Japan). The primers specific for *rad51* were 5'-GATGGTGGTATTGAATCTGGTTCAATACTG and 5'-GACGGCAACACCAAACTCATCGGCTAATCT. The nucleotide sequences of primers for *recA* and *actin15* are described in previous reports (Akaza et al., 2002; Hasegawa et al., 2004). DNA fragments were amplified 14-24 cycles after reverse transcription and run through an agarose gel to compare signal intensities.

UV light sensitivity assay

CM and CM(UV) were prepared from dish cultures as described above. Equal numbers of *D. discoideum* cells were incubated with CM or CM (UV) for 40 min. Cells were then washed with the phosphate buffer and irradiated by 200-J/m^2 UV

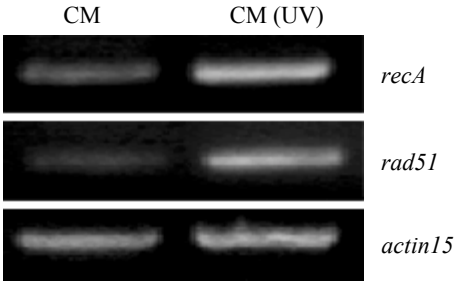

Fig. 3. Expression of *recA* and *rad51*. Expression levels of *recA* and *rad51* were analyzed by RT-PCR as described in Materials and Methods. The reaction products of *recA* (18 cycles), *rad51* (16 cycles) and *actin15* (14 cycles) are presented.

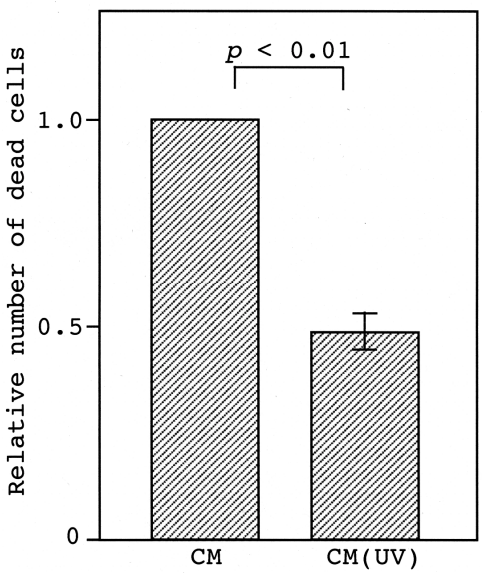

Fig. 4. Dead cells in CM or CM(UV). Cells incubated with CM and CM(UV) were exposed to UV light as described in Materials and Methods. Dead cells that were stained by phloxine B and viable cells were counted under a microscope. Three sets of experiments were carried out.

light. Then the cells were incubated with a fresh HL5 medium for 20 h. Dead cells that were stained by phloxine B and viable cells were counted under a microscope as described in a previous report (Garcia et al., 2000).

RESULTS AND DISCUSSION

D. discoideum exposed to UV light increased motility

Experiments were carried out to examine whether *D. discoideum* would increase motility when exposed to UV light. *D. discoideum* cells were placed on a glass slide with a portion of HL5 medium for 20 min. The liquid medium was removed and the cells were exposed to UV light (50 J/m^2). Irradiation of 50 J/m^2 is a sublethal condition for *D. discoideum* (Garcia et al., 2000; Hasegawa et al., 2004). Soon after the irradiation, a fresh medium was placed on the cells. Unirradiated cells migrated at 4.5 $\pm$ 0.7 μm/min, while UV irradiated cells migrated at 19.4 $\pm$ 1.7 μm/min (Fig. 1). The p value obtained from a t test was less than 0.01. The results indicate that UV irradiation increased the motility of the cells. Increase in motility would be important for *D. discoideum* to take refuge from DNA-damaging agents.

UV light-irradiated *D. discoideum* released a factor which increased motility in unirradiated cells

Refuge from a damaging agent prior to exposure would be of great benefit for organisms because organisms that have escaped prior to exposure to an agent are able to survive and grow at

a place of refuge. Exposed organisms might therefore release a signal to increase motility in unexposed organisms. Unexposed organisms would be able to escape from the agent and survive at the expense of exposed organisms. To determine whether *D. discoideum* releases such a signal, the following experiments were carried out. A medium in which UV light-irradiated cells had been incubated for 20 min was removed and used as a conditioned medium, CM (UV). Cells that had not been exposed to UV light were incubated with the CM (UV) and observed under a microscope. The migration speed of these cells increased remarkably (29.7 $\pm$ 2.8 μm/min) even though they had not been exposed to UV light (Fig. 2). For a control, a medium in which unexposed cells were incubated for 20 min was removed as a conditioned medium, CM. Migration speed of cells in the CM was 6.2 $\pm$ 0.7 μm/min (Fig. 2). The results of a *t* test ($p > 0.05$) showed that this value was not different from the value obtained from the cells in HL5 medium (4.5 $\pm$ 0.7 μm/min). These results indicate that a factor that significantly enhanced motility was released from UV light-irradiated *D. discoideum* but not from unirradiated cells.

Released factor decreased UV light sensitivity of *D. discoideum*

It has been observed that *D. discoideum* increases expression of *recA* (by 16 fold) and *rad51* (by 10 fold) following exposure to 50-J/m^2 UV irradiation (Hasegawa et al., 2004 and unpublished result). The following experiment was carried out to examine whether the factor in CM(UV) would induce expression of these genes in unirradiated cells. *D. discoideum* cells incubated with CM or CM(UV) were collected to extract total RNA for RT-PCR analysis. The RT-PCR products were resolved by gel electrophoresis and their signal intensities were analyzed. Expression of *actin15* was analyzed as an experimental control as described in previous reports (Akaza et al., 2002; Hasegawa et al., 2004). Expression levels of *recA* and *rad51* in the cells in CM(UV) were higher (~3-fold) than those in the cells in CM. These results indicate that a factor in CM(UV) induced DNA repair enzymes in cells even though the cells were not exposed to UV light (Fig. 3).

The next experiment was performed to determine whether the induction of DNA repair enzymes contributes to the decrease in UV sensitivity of *D. discoideum*. Equal numbers of cells were incubated with CM or CM(UV) for 40 min, irradiated by 200-J/m^2 UV light and then incubated with a fresh HL5 medium for 20 h. Dead cells were counted following dye staining. Relative number of dead cells is shown with the result obtained from a *t* test (Fig. 4). The UV light sensitivity of *D. discoideum* that had been incubated with CM (UV) decreased (by 0.49 $\pm$ 0.07 fold) compared with that of the cells that had been incubated with CM.

The experimental evidence presented here demonstrates that UV light-irradiated *D. discoideum* released a factor that increased motility and induced DNA repair enzymes in unirradiated cells. This factor would be important for *D. discoideum* to alleviate damage and avoid annihilation in an emergency. Molecular identification of the factor is in progress.

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