**Short communication**

**Oxidative DNA Damage Induced by 364-nm UVA Laser in Yeast Cells**

Kazuo Negishi\(^1,5\), Shoichi Higashi\(^2\), Takanori Nakamura\(^2\), Chie Otsuka\(^3\), Masakatsu Watanabe\(^2\) and Tomoe Negishi\(^4\)

\(^1\)Okayama University Advanced Science Research Center and \(^4\)Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

\(^2\)Okazaki National Research Institutes, National Institute for Basic Biology, Okazaki, Japan

\(^3\)Research Institute for Biological Sciences, Okayama, Japan

(Received March 10, 2006; Accepted April 19, 2006)

The mechanisms of the toxic effects of UVA (320–400 nm) irradiation remain unclear. The actions of monochromatic longer wavelength UVA, in particular, have been difficult to analyze because of a lack of a powerful light source; however, a UVA laser that can be used for biological studies was recently developed. In the current studies, we examined the effects of 364-nm irradiation on yeast cells using a potent UVA laser. We found that, when irradiated under aerobic conditions, yeast cells lacking Ogg1 glycosylase were more sensitive than those with Ogg1. The ability of the 364-nm light to kill the yeast cells was almost eliminated by purging with argon gas. The mutagenic effects of the UVA irradiation did not appear to be enhanced by a lack of Ogg1. These results indicate the killing of yeast cells by 364-nm UVA may be dependent on oxidation and may involve DNA lesions that can be repaired by Ogg1.

Key words: UVA, Ogg1, oxygen, laser, yeast

**Introduction**

UVA (320–400 nm) light is carcinogenic and mutagenic (1–3). The mutagenic effects of UVA are thought to involve both oxidative damage (4,5) and the formation of bi-pyrimidine photoproducts (6,7), although the mechanism remains unclear. Currently available data in eukaryotes indicate that UVA-induced mutagenesis is mediated by oxidative damage in yeast (5) but by bi-pyrimidine photoproducts in mammalian cells (6,7). In addition, UVA includes a wide range of wavelengths that may have different effects, although it has been difficult to study the effects of narrow-range or monochromatic UVA, especially for longer wavelength UVA, because a strong UVA source has not been available for biological systems. Recently, however, long-wavelength UVA (364 nm) lasers have been developed. In the present study, we used a laser to analyze the mutagenic and toxic effects of 364-nm UVA irradiation on yeast cells that express or lack Ogg1 glycosylase.

**Materials and Methods**

**Yeast strains:** *Saccharomyces cerevisiae* strain B7528 (MATa cyc1-31 cyc7-67 lys5-10 ura3-52) (8) and its *ogg1* disruptant were used in this study. To disrupt the *OGG1* gene, we transformed B7528 by electroporation (9) with a plasmid in which the *ogg1* gene is disrupt-ed by insertion of *URA3* (generously provided by Prof. K. Yamamoto and Dr. T. Nunoshiba, Tohoku University, Sendai, Japan). *Ura\(^+\)* transformants were selected, and the insertion in the *OGG1* gene was confirmed by Southern blotting. The strain showed an elevated rate of spontaneous mutation as expected from previous reports of other Ogg1-deficient strains (10). Strain B7528 is suitable for studying biological damage by oxygen. Oxygen depletion in this strain is not expected to be harmful because they do not utilize oxygen for their growth.

**Laser apparatus:** For irradiation, we used a 364-nm argon laser (Hitachi High-tech, Inc., Tokyo, Japan) with a maximum power of 2 W. An area of 5.4 × 5.4 cm was homogeneously irradiated with a vertical beam. Details of the apparatus will be published elsewhere (S. Higashi et al.).

**UVA irradiation:** After culturing at 30°C for approximately 15 h from a few colonies, yeast cells were collected by centrifugation, washed once with water, and resuspended in water. Dose dependence studies using two strains were performed in 96-well microtiter plates. To count surviving cells, aliquots of cell suspensions were diluted 10\(^4\)-fold, plated onto YPD plates, and counted after 2 to 3 days. To score mutants, 40 μL of...
the irradiated suspension was plated onto plates containing a SD medium supplemented with uracil (20 mg/L), lysine (30 mg/L) and canavanine (60 mg/L), and counted 4 to 5 days after plating. To compare aerobic and anaerobic conditions, cells were irradiated in quartz cuvettes with a 1-mm light path. For anaerobic irradiation, a portion of the yeast cell suspension was purged with argon gas for 30 min and then transferred with a syringe to a cuvette to which a balloon filled with argon was attached.

**Results and Discussion**

We first analyzed the survival of yeast cells after irradiation with the 364-nm UVA laser. As shown in Fig. 1, Ogg1-expressing yeast cells started to die when irradiated at intensities higher than 400 kJ/m². In contrast, Ogg1-deficient cells were inactivated according to the logarithm of the intensity. This indicates that base excision involving Ogg1 glycosylase may eliminate toxic damage caused by the UVA irradiation.

This involvement of Ogg1 suggests that the damage is oxidative in nature. To determine if this is correct, we extensively purged a portion of the yeast cell suspension with argon gas to remove dissolved oxygen. When purged and control cells were irradiated with the laser, inactivation took place only in the control cells (Table 1). This confirmed that the damage was dependent on oxygen. Kozmin *et al.* reported that UVA sensitivities of Ogg1-expressing and -deficient yeast strains are similar (5), although they used a broad-spectrum UVA source. One possible explanation for these contradictory results is that shorter UVA wavelengths have oxygen-independent toxic effects that cannot be repaired by Ogg1 and that mask oxygen-dependent toxic effects at longer wavelengths that can be repaired by Ogg1. Indeed, in Drosophila, there are differences in the toxic effects of longer and shorter wavelengths of UVA (11).

In contrast, mutations induced by the laser UVA irradiation were not substantially enhanced by the absence of Ogg1 glycosylase (Fig. 2). Kozmin *et al.* reported highly efficient induction of mutagenesis by UVA in Ogg1-deficient yeast (5). In our case, the irradiation at 364 nm seems to be mutagenic, but the mutagenic responses of the two strains did not differ much. Again, the contradictory results could be due to
the differences in the wavelength of UVA irradiation. However, the mutation rates induced by the laser light might be too low to compare differences between the strains.

In Drosophila larvae, irradiation with the 364-nm laser induces the formation of 8-hydroxyguanine lesions in DNA (T. Negishi et al., unpublished); however, this may not be a major toxic lesion if it is highly mutagenic in the Ogg1-deficient strain as suggested by Kozmin et al. (5). Other DNA lesions, including 8-hydroxyadenine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy), can be repaired by Ogg1 (12,13). Ring-opened lesions like Fapy are known to inhibit DNA synthesis and may be highly toxic (14,15). Alternatively, the irradiation may cause the formation of 8-hydroxyguanine that may be toxic rather than mutagenic due to the specific locations in which it was formed.

Our present study showed that the UVA laser is a useful tool for evaluating and analyzing the effects of irradiation with UVA at 364 nm. We found here that the effects of the 364-nm irradiation are oxygen-dependent. Further studies using the laser will help elucidate the complex mechanisms of how oxidative damage is produced by UVA irradiation at 364 nm.

Acknowledgements: We thank Ms. Asako Kawakami for her technical assistance. This work was supported by a Grant-in-Aid for Scientific Research (C) (No. 17590060) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. This study was carried out under the NIBB Cooperative Research Program for the Okazaki Large Spectrograph (5–511).

References
3. Cadet J, Sage E, Douki T. Ultraviolet radiation-mediated damage to cellular DNA. Mutat Res. 2005; 571: 3–17.