Photoreactivation of UV damage in cultured *Drosophila* cells

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Summary. Cell survival and photoreactivation of 254 nm ultraviolet (UV) light damage in a wild type *Drosophila* cell line was assayed by colony formation in liquid medium. F_o, F_q, and extrapolation number for the exponential portion of survival curves are 21 J/m^2, 3.6 J/m^2, and 1.5 for non-photoreactivated cells and 110 J/m^2, 11.2 J/m^2, and 1.3 for those exposed to photoreactivating light. Maximal photoreactivation occurs at the 100 J/m^2 region of the curve. At 10 and 50% survival, 75–80% of the UV damage was photoreactivable.

Key words. Cell survival; photoreactivation; insect cell; *Drosophila;* colony formation; DNA repair; ultraviolet radiation.

Cells from *Drosophila melanogaster,* a dipteran insect, have been more widely studied than cells from other insects and a great deal is known about their genetics and biochemistry[1]. Cultured *Drosophila* cells have been observed to be a few times more resistant to the lethal effects of ionizing radiation than cultured mammalian cells, but much more sensitive (on the order of 13 times) than cultured lepidopteran insect cells[2]. At least one line of lepidopteran cells, TN-368, is also quite resistant to the lethal effects of 254 nm ultraviolet (UV) light and is proficient at repairing UV-induced damage by photoreactivation (PR)[3]. Two mutant *Drosophila* cell lines derived from flies exhibiting a severe reduction in meiotic recombination have been examined with respect to survival and PR of UV damage[4]. However, survival in this study was assayed by cloning in agarose, a stressful condition for cultured insect cells[5] and although PR was assessed qualitatively, PR fluences and detailed quantitation of survival parameters after PR were not determined.

The present studies were undertaken to determine the ultraviolet light survival response of a wild type *Drosophila* cell line by colony formation in liquid medium and to quantify the ability of these cells to photoreactivate UV-induced damage. These experiments were performed in the wild type *Drosophila* cells to ascertain their relative resistance to UV-light compared to the lepidopteran TN-368 line and the mutant *Drosophila* lines, and because of the copious amount of information concerning the genetics and biology of *Drosophila* for correlative purposes.

Materials and methods. The WR69-DM-1 cells (Schneider's line 1) are a continuous line of *Drosophila melanogaster* cells derived by enzymatic dissociation of Oregon-R embryos on the verge of hatching[6]. Details concerning the cells and culture conditions can be found elsewhere[3]. Four 15-W germicidal lamps were used to perform 254 nm irradiations. Cells were irradiated at a fluence rate of 1.3–1.4 J/m^2/s. Photoreactivations were accomplished using two 15-W black lights at a fluence rate of approximately 5 J/m^2/s. A piece of glass, 4 mm in thickness, was placed between the lights and the plates to help prevent the cells from heating and to eliminate far-UV wavelengths. Except for photoreactivation treatments, all cellular manipulations were performed in the dark or in the presence of dim yellow incandescent light when necessary. Exponentially growing cells were used in all experiments. Cells were grown in 60-mm plates, the medium was poured off and replaced with 2 ml of a modified (pH and osmotic pressure adjusted) Hanks' Balanced Salt Solution (HBSS), and the buffer aspirated with a 1-ml pipet. In addition, cells growing within a few millimeters of the periphery of the plate were aspirated to insure that, in the event shadowing of the UV by the sides of the plate occurred, no cells would be affected and included in the assay. Approximately 0.15 ml HBSS was

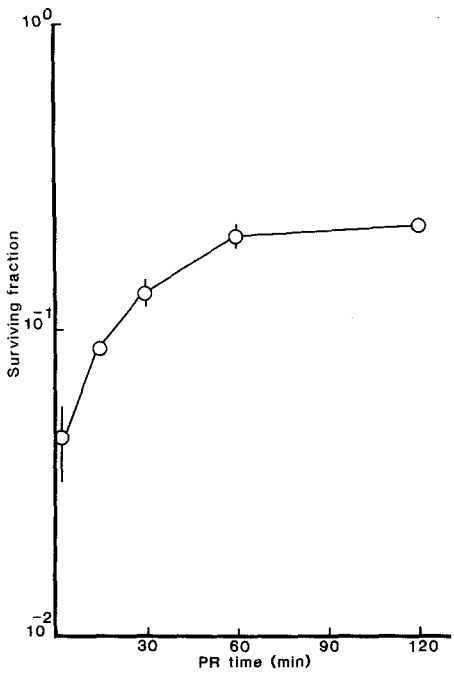

Figure 1. Time course of photoreactivation of WR69-DM-1 cells at a photoreactivating fluence rate of approximately 5 J/m^2/s. Initial 254 nm fluence was 100 J/m^2. Points represent the mean ± SE of 10 replicates from 2 experiments.

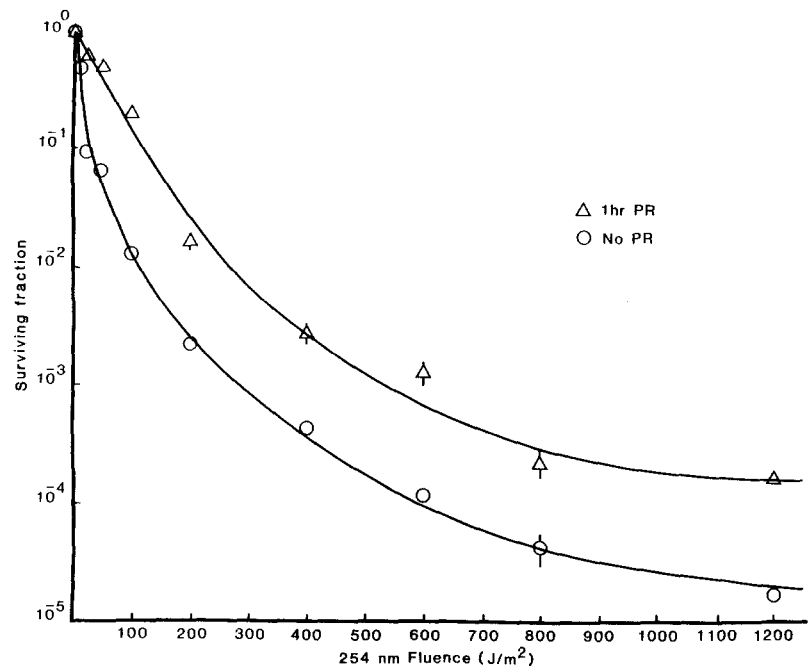

Figure 2. 254 nm UV dose-survival curves for otherwise untreated WR69-DM-1 cells and for those exposed to 60 min of photoreactivating light. Each point represents the mean ± SE of 2–5 experiments with 5 replicates per experiment.

then gently added to prevent cells from drying during long UV exposures. For short irradiation times, results were not distinguishable from those where no HBSS was added to the plates. The cells were then irradiated with 254 nm light after removing the lid from the plate. For cells receiving photoreactivation, 2 ml spent medium (HBSS could be substituted for spent medium with very little difference in results) were added and the plate placed under black lights. Temperature was maintained at 26–28 °C during irradiation and black-light exposure. Following black-light exposure, plates were handled in the same manner as those receiving 254 nm radiation only. The cells were removed from the plates, diluted, plated into replicate 60-mm dishes containing approximately 4 ml medium and 5×10^5 feeder cells which had been γ-irradiated with approximately 338 Gy (No discernable difference was observed when feeder cell number was varied to keep total cell number per plate constant.), and incubated in the dark for fourteen days at 28 °C to allow for colony formation[2]. The surviving colonies were stained and counted, and cell surviving fractions were calculated as previously described[7]. Briefly, the number of colonies in untreated control plates was used to determine the absolute plating efficiency for each experiment by dividing the number of colonies by the number of cells plated. A similar calculation was performed for each UV treatment to obtain a plating efficiency. This plating efficiency divided by the absolute plating efficiency of the control cells yields the relative plating efficiency which is plotted as surviving fraction in the figures. The absolute plating efficiency of the WR69-DM-1 cells during these experiments averaged approximately 38%.

Results. The relationship between the amount of exposure to photoreactivating light and the degree of photoreactivation is presented in figure 1. The photoreactivated cells had previously been irradiated with a 254-nm fluence of 100 J/m^2. The kinetics of PR at the PR fluence utilized were slower than for lepidopteran cells with approximately 26% completion in 15 min, 54% completion in 30 min, and 96% completion obtained by 60 min[3]. Based upon this information, 60 min was used for all experimental PR times. At the dose rate used, 5 J/m^2/s, the 60-min exposure is equivalent to a total photoreactivating fluence of 18 kJ/m^2. This total fluence is approximately 1.5 times that providing maximal PR for TN-368 lepidopteran cells[3] and chick-embryo fibroblasts[8]. For the experimental conditions used, PR treatment alone had no significant effect on cell survival.

The survival response of the WR69-DM-1 cells to 254 nm radiation, in both the absence and presence of photoreactivating light, is depicted in figure 2. The cells are more resistant to the UV treatment than mammalian cells, a fluence of 100 J/m^2 being required to reduce survival to approximately 1.3×10^{-2}. When the 100 J/m^2 is followed by PR, survival is reduced to only about 2.0×10^{-1}. This is nearly 16 times greater than without PR. The increase in survival due to PR varies with fluence from 5.2 to 15.6 times the survival for cells treated with UV alone. The photoreactivable or PR sector, that fraction of the UV damage that is photoreactivable, was calculated as $1 - \frac{F_{(-PR)}}{F_{(PR)}}$, where $F_{(PR)}$ and $F_{(-PR)}$ are, respectively, the fluences resulting in equivalent survival levels with and without photoreactivation[9]. The PR sectors of UV damage for the *Drosophila* cells at 10 and 50% survival are 0.76 and 0.79, respectively. This compares with PR sectors at the same survival levels of 0.68 and 0.65 for the TN-368 cells[3].

Survival curves, both with and without photoreactivation, have a very small initial shoulder leading to an exponential region after which the curves continue to decrease but with a decreasing slope. Both curves have the same general shape and similar extrapolation number, but the slope of the exponential portion of the PR curve is shallower than that of the curve without PR. A regression line was fitted to the points in the exponential region (fluences of 10–25 J/m^2 for No PR and 25–200 J/m^2 for PR) to determine the F_o, F_q, and extrapolation number of each curve; F_o and F_q being analogous to D_o and D_q for ionizing radiation survival curves. F_o, F_q, and extrapolation number are 21 J/m^2, 3.6 J/m^2, and 1.5 for non-photoreactivated cells and 110 J/m^2, 11.2 J/m^2, and 1.3 for those receiving photoreactivating treatment.

Discussion. These studies clearly demonstrate that the well-characterized WR69-DM-1 *Drosophila* cell line, which is a few times more resistant than most mammalian cells to the lethal effects of ionizing radiation, is also more resistant to 254 nm UV radiation. In addition, it is apparent that the cells are quite competent to repair UV-induced damage by photoreactivation. At 21 J/m^2, the *Drosophila* cell F_o is considerably higher than that of two mutant *Drosophila* cell lines[4], rat kangaroo cells[10], chick-embryo cells[8], fish cells[11], frog cells[12,13], and several other prokaryotes[14], but nearly four times lower than the F_o for the TN-368 lepidopteran insect cell line[3].

The effect of photoreactivation on the shape of the *Drosophila* survival curve is to extend a very small shoulder and decrease the slope (fig. 2). This is the more typical effect of photoreactivation on survival[11,13,14], although Griggs and Bender[12] reported an increase in extrapolation number with PR of frog cells, but no change in slope. The extrapolation number of the *Drosophila* cells is approximately the same in the absence and presence of photoreactivating light. The F_o, which can be used as a measure of UV resistance, is about 5 times greater in photoreactivated over non-photoreactivated WR69-DM-1 cells (fig. 2). In addition, for a given fluence, survival can be increased nearly 16 times by photoreactivation. These data demonstrate that the *Drosophila* cells are quite proficient at repairing UV damage by PR and that nearly 75–80% (PR sector of 0.76–0.79) of the damage is photoreactivable. PR is specific for the repair of pyrimidine dimers. Therefore, these results suggest that the primary lethal lesion induced in the *Drosophila* cells by 254 nm light is the pyrimidine dimer.

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