Ultraviolet Irradiation of the Vegetative Cells of

Dictyostelium discoideum

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Experiments on the effect of ultraviolet (UV) light on the survival of vegetative Dictyostelium discoideum cells indicate that this is a relatively UV-resistant organism. Several factors suggest the presence of some type of repair process. Experiments to test for liquid-holding recovery and simple photoreactivation yielded negative results. Acriflavin and caffeine were utilized to possibly interfere with dark repair. Acriflavine produced no UV sensitization, but caffeine did cause a concentration-dependent decrease in survival of irradiated cells. When UV-irradiated cells were illuminated with photoreactivating light while suspended in caffeine, the survival increased above that for cells treated with caffeine alone, suggesting an overlap between lesions repaired by photorepair and dark repair. Growth experiments showed that UV light induced a dose-dependent division delay, followed by a period of retarded growth characterized by the presence of a constant fraction of nonviable cells in the irradiated population. The delayed exposure of cells to caffeine after irradiation showed that the magnitude of the caffeine sensitization diminished throughout the division-delay period. An action spectrum indicated probable nucleoprotein involvement in the induction of division delay. UV light retarded ribonucleic acid and protein synthesis and temporarily blocked deoxyribonucleic acid synthesis. However, synthesis of all three accelerated prior to the end of the division-delay period and then closely paralleled the increase in cell number.

Insight into the basic molecular processes underlying the complex phenomena of biological differentiation and development is often obtained from model systems. One relatively simple microbial model system is the cellular slime mold, Dictyostelium discoideum (2, 8, 32, 37). This eukaryotic organism, which feeds on bacteria, has a vegetative amoeboid growth phase with about a 3-hr cell doubling time and a developmental stage triggered by starvation. During this development, differentiation into two cell types, stalk cells and spore cells, occurs in the absence of cell multiplication. Unlike the true slime molds, these cells do not fuse into a common cytoplasm, but do aggregate into multicellular masses near the beginning of development.

One way to gain further knowledge of the molecular processes involved in these transformations is to subject the system to specific stresses under various circumstances and at different growth stages and analyze the responses. One such stress, whose molecular action is at least partially understood, is ultraviolet (UV) light (12, 26-28). The use of UV irradiation to alter the processes associated with differentiation and development in D. discoideum may be informative. It is also hoped that such studies will further clarify the basic mechanisms of UV action on cells in general.

Other than the use of UV to produce developmental mutants (33), no prior information is available on the responses of D. discoideum to this agent. Here we report the results of our initial experiments on UV light action on the vegetative cells of D. discoideum. These include UV dose-effect curves for clone formation, UV-induced division delay and postirradiation growth effects, an action spectrum for division delay, photoreactivation studies, and the effects of some chemical agents believed to alter repair of UV damage in other systems.

MATERIALS AND METHODS

D. discoideum strain NC-4 (a stable haploid) was used throughout this work (34). The cells were grown in association with Escherichia coli B/7 on either the solid plating medium (BPM) described by Bonner (1) or in a liquid bacterial suspension (bacterial concentration approximately 1010 cells/ml) in a

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manner similar to that given by Hohl and Raper (11). The growth temperature at all times was 23 C. The solution used for the suspension and dilution of the D. discoideum cells as well as for the washing and suspension of the bacteria used to support liquid growth was a Tris salt solution (TSS) containing 4.85 g of tris(hydroxymethyl)aminomethane, 0.75 g of KCl, and 0.60 g of NaCl per liter of water, with the pH adjusted to 7.0 with HCl. For irradiation experiments, the cells were grown in liquid suspension with bacteria, harvested during the log phase of growth, and washed free of external bacteria by repeated low-speed centrifugation (150 X g for 2 min). They were then suspended in TSS at the concentration desired for irradiation, usually 1.5 X 10^9/ml. UV irradiations were done with a high-intensity water prism monochromator similar to that of Fluke and Setlow (7), with a Philips SP 500W high-pressure Hg arc lamp. The incident intensity was measured with a photocell whose output was determined by a vacuum-tube voltmeter; this arrangement was calibrated by a thermopile previously calibrated with a standard lamp. The vegetative D. discoideum cells were irradiated in a 1-cm-path quartz cuvette containing a magnetic stirring bar to provide mixing during irradiation. It was necessary to use the Morowitz correction (19) to obtain the average intensity throughout the sample. A bank of General Electric BLB Black Lights was used in all photoreactivation experiments. Care was taken to eliminate any possible short-wavelength UV. All irradiations were done at 23 C.

Survival of the irradiated D. discoideum cells was determined by their ability to undergo the necessary number of divisions to form a "plaque" (31) in the dense bacterial lawn. Cells were irradiated, diluted in TSS, plated on BPM plates in association with 0.1 ml of washed bacteria at 10^8/ml, and incubated at 23 C. Plaques began to appear in about 3 days. When acriflavine (Allied Chemical, Morristown, N.J.) and caffeine (Calbiochem, Los Angeles, Calif.) were used in BPM plates, these chemicals, made up in concentrated stock solutions, were not added until the plating medium had been autoclaved.

In experiments designed to determine the effects of radiation on cell division, growth, and macromolecular synthesis, washed D. discoideum cells were irradiated in 2.5-ml portions, added to a TSS bacterial suspension, and allowed to grow at 23 C with stirring. The growth of irradiated populations was determined by hemacytometer and plaque counts. Chemical assays were used for the measurement of macromolecular synthesis. Samples were periodically removed from growing cultures of irradiated cells, washed free of external bacteria by centrifugation, and the amount of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein per sample was determined. The orcinol reaction as given by Brown (3), the Keck modification of the Ciernioti-indole-hydrochloride reaction (15), and the Folin reaction as given by Lowry et al. (17) were used to determine RNA, DNA, and protein, respectively. Because of the high RNA to DNA ratio in D. discoideum (about 70:1), it was necessary to separate the three components before assay, by a method similar to that given by Schmidt and Thannhauser (25), to prevent a large positive indole-hydrochloride reaction with the cellular RNA.

RESULTS

The clone-forming ability ("plaques") of UV-irradiated vegetative D. discoideum cells is shown in Fig. 1 ("No Caffeine" curve). The plaques formed by the nonirradiated samples were of uniform size and all appeared at approximately the same time (first visible at about 2.5 to 3 days). The plaques formed by the irradiated samples were characterized not only by a wide size range but also by a delayed appearance; some plaques were not visible until 48 hr after the appearance of the first plaques. Both the delay of plaque appearance and the diversity of size were dose-dependent.

To detect any liquid-holding recovery (23), cells were irradiated in the normal manner, diluted into either TSS or nutrient broth, with no bacterial food source, held at 4 or 23 C, and plated after a holding period of 0 or 4 hr. Regardless of the condition employed, no significant difference could be detected in either cell survival or plaque morphology.

![Fig. 1. Survival of colony (plaque)-forming ability as a function of incident 265-nm UV dose, with no caffeine in plating medium (●) or with 0.05 (△), 0.12 (●), 0.15 (□), or 0.20 (■) mg of caffeine per ml.](http://journals.asm.org/figure/010207000376527a.png)
The use of acriflavin (6, 27) or caffeine (18, 22, 24) to sensitize cells to UV radiation is sometimes useful in demonstrating the possible existence of dark repair. For the former agent, irradiated cells were immediately diluted into TSS containing the proper concentration of acriflavin, and plated on BPM plates containing acriflavin. All acriflavin experiments were done in dim light. The acriflavin produced no UV sensitization for concentrations up to 8 \times 10^{-4} \text{ mg/ml}. The primary effect of acriflavin was to slow the growth of both the control and the irradiated cells, resulting in small plaques which became increasingly difficult to detect as the acriflavin concentration was increased. At concentrations above 8 \times 10^{-4} \text{ mg/ml}, a small apparent increase in sensitivity was detected, probably due to poor plaque visibility.

The effect of caffeine on UV survival is shown in Fig. 1. The experimental procedure was identical to that described above for acriflavin. As can be seen, caffeine does produce a definite concentration-dependent effect on the survival of irradiated cells. A caffeine concentration of 0.15 \text{ mg/ml} was sufficient to remove the shoulder from the survival curve. Higher concentrations would probably have only the effect of increasing the sensitivity, as the single datum point for 0.20 \text{ mg/ml} indicates. Concentrations up to 0.20 \text{ mg/ml} had no effect on the plaque-counting efficiency of nonirradiated controls, but when irradiated samples were subjected to concentrations above 0.20 \text{ mg/ml}, the plaques became difficult to count because of their small size.

Postirradiation illumination of the cells with potentially photoreactivating light did not produce an increase in survival (top curve, Fig. 2). In fact, at the highest illumination dose used (7.5 \times 10^{4} \text{ ergs/mm}^2), the survival of both control and irradiated samples showed a slight decrease. This apparent lack of photoreactivation suggested that the cells contained no photoreactivating enzyme, that induced damage was not photoreparable, or that photorepair was masked by overlap with an efficient dark repair.

This last possibility was tested by illuminating irradiated cells with photoreactivating light while suspended in TSS containing caffeine at a concentration of 0.15 \text{ mg/ml}, and then plating them on BPM plates containing the same caffeine concentration. Since it has been reported that caffeine does not inhibit photoenzymatic repair (18), this combination should have the effect of blocking dark repair while allowing photorepair, if present, to be expressed (Fig. 2). Although not large, the photoinduced survival increase in the presence of caffeine was repeatable and can be attributed to some form of photoreactivation.

Postirradiation growth in liquid suspension was measured by a series of hemacytometer counts, which gave the total cell count, including both viable and nonviable cells, and by plating on BPM plates, which gave the viable count only. Cells were plated with and without caffeine as a function of time after the start of the postirradiation incubation period. Some representative results are shown in Fig. 3 for doses of 770 and 1,330 \text{ ergs/mm}^2. In each case, the curves from top to bottom are: hemacytometer and plaque counts for the unirradiated control, indicating 100\% plating efficiency and exponential growth; hemacytometer count of the irradiated culture, indicating total cell number; viable cell counts from platings in the absence of caffeine; viable cell counts from platings in the presence of caffeine. The growth as determined by hemacytometer counts clearly indicates an initial period during which there is no increase in cell concentration, whereas the control has increased in the normal exponential manner. The viable cell count, although lower than the total because of the irradiation, also remained constant during this time. The duration of the UV-induced division delay was dependent upon the incident radiation dose (see Fig. 4). It was also observed microscopically that the average cell size increased during this period, the maximum occurring just prior to the resumption of cell division. After the period of division delay, the cells resumed growth at a rate somewhat lower than normal. Not only was there a lag in growth of the total population, but there was also an equal lag for those cells which are classed as survivors by virtue of their "plaque"-forming ability (Fig. 3).

A UV-induced division delay could be a period during which repair processes were operative. To test for this possibility, the plaque counts after irradiation were measured on BPM plates containing 0.15 \text{ mg of caffeine per ml}. The results (lower curve in Fig. 3) show that the cells became immune to the sensitizing effect of caffeine during the course of the division delay. The effect of caffeine almost completely disappeared by the time that growth resumed.

The effect of several representative wavelengths on the duration of the radiation-induced division delay is shown in Fig. 4. The lag is linear with dose up to 1,500 \text{ ergs/mm}^2. Figure 5 (solid curve) shows an action spectrum for the division lag. Light of 275-nm wavelength is the most effective for the induction of division delay. D. discoid-um contains a very large amount of RNA and protein. If it is assumed (maybe incorrectly) that the site of the UV action for division delay is in the nucleus, then much of the incident ra-
radiation will be absorbed before reaching that site. From the absorption spectrum of lysed cells and the known cell volume, it was possible to estimate to what extent each wavelength was absorbed before reaching the nucleus. The action spectrum in terms of the estimated dose incident upon the nucleus is shown as the dotted line ("Corrected") in Fig. 5. The relative effectiveness is increased and the peak shifted to shorter wavelengths. For comparison purposes, an absorption spectrum (relative scale only) for a typical DNA is shown as the dashed line (Fig. 5; reference 12).

Some results of the macromolecular synthesis studies are shown in Fig. 6. The dashed lines indicate the relative hemacytometer counts for the same samples. In each case, the synthesis was retarded by the radiation, the degree of retardation being a function of both the incident dose and the particular macromolecule. The DNA is more affected than is RNA or protein. This DNA synthesis was characterized by an initial dose-dependent lag, followed by a period of accelerated synthesis before the onset of cell division. Finally, in the latter stages of the division delay and during the period of reduced growth, the rate leveled off somewhat and then paralleled the rate of total cell increase. Figure 7 shows the ratio of the macromolecular content per irradiated cell to that of an unirradiated cell at various times after irradiation. The arrow (Fig. 7) indicates the end of the division delay. For all components this ratio increased, was maximal at approximately the time cell division began, and then approached
more than are RNA synthesis and protein synthesis.

**DISCUSSION**

*D. discoideum* vegetative cells are relatively resistant to UV. The shoulder on the survival curve and the growth pattern of the damaged cells are suggestive of a possible radiation repair process. The repair could occur during the division lag produced by UV even under the usual nutrient conditions and, hence, holding under non-nutrient conditions (no bacteria) to test for liquid-holding recovery would not be expected to give any additional survival increase. None was observed.

Acriflavine did not act as a sensitizer. The maximal concentration used here (8 × 10⁻⁴ mg/ml) corresponded approximately to those used with mammalian cells (22), *E. coli* (36), and *H. influenzae* (20). Although the latter two organisms showed large decreases in survival, Rauth (22) was unable to produce a sensitization in the mammalian cell line even at concentrations sufficient to decrease the control survival by 50%. Higher acriflavine concentrations have been required to inhibit dark repair in organisms more complex than bacteria. Davies (5) used concentrations of 8 × 10⁻³ to 15 × 10⁻³ mg/ml to inhibit dark repair in spores of *Chlamydomonas reinhardtii*. Coohill and Deering (4) used 50 × 10⁻³ to 100 × 10⁻³ mg/ml in *Blastocladia emersonii* to obtain only a small effect. The absence of an acriflavine effect in *D. discoideum* and in mammalian cells does not seem to result from an inability of the acriflavine to enter the cell, since it did exert a large effect on the growth of nonirradiated controls.

Caffeine produced a concentration-dependent decrease in survival of irradiated cells. The fact

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**Figure 5.** Action spectrum in terms of division lag for 1,100 ergs/mm² as a function of wavelength. Measured in terms of incident dose (○); estimated in terms of dose reaching cell nucleus (⋯); DNA absorption spectrum for comparison (-- --).

**Figure 6.** UV effect on macromolecular synthesis as determined by measuring the amount of RNA, DNA, and protein present per ml of sample. Concentration versus time for unirradiated (●) and irradiated (Δ) samples. Dashed lines show hemacytometer count of total cell growth for same experiment. (To obtain the concentration in micrograms per milliliter, multiply the graph values by 0.34 for DNA, 24 for RNA, and 50 for protein.)

1.0 as growth continued. This is consistent with the increase in cell size observed by microscopic examination during the period of division delay. Figure 7 illustrates that DNA synthesis is affected

**Figure 7.** Ratio of macromolecular content per irradiated cell to that in an unirradiated control cell as a function of time after irradiation at three UV doses. Arrows indicate end of division lag period as determined by hemacytometer counts.
Photoreactivating light alone had no effect on the survival of the UV-irradiated *D. discoideum* cells. When the irradiated cells were exposed to photoreactivating light in the presence of caffeine, some photoreactivation occurred. It is not known whether direct enzymatic photorepair or some light-stimulated indirect repair (14) was responsible for the increased survival. However, it is known that the illumination doses employed here did not induce a division delay in non-UV-irradiated controls nor measurably alter the duration of the UV-induced division delay. It appears as if dark repair overlaps photorepair under the conditions of these experiments. Such overlap has been reported for V-gene repair and photorepair by Harm (10), although neither completely overlaps the other. Metzger (18) claims a complete overlap between the lesions in irradiated phage which are repaired by dark- and photorepair mechanisms in *E. coli* hosts. Since different organisms exhibit different action spectra for photoreactivation (13, 16, 29), wavelengths other than those used here might produce more efficient photoreactivation.

UV light produced a distinct dose-dependent division delay in these cells. The widespread size variation and delayed appearance of the plaques formed by irradiated cells can be explained by the division delay and the reduced rate of growth which follows. The experiments of Fig. 3 indicated that the total population after irradiation is not composed of two components, one that ceases division entirely ("dead" cells) and one that continues to divide normally ("survivors"). Instead there appears to be a constant fraction, not number, of nonviable cells present in the irradiated sample. The actual number of nonviable cells increases during the reduced growth rate period. This means that a certain proportion of daughter cells arising from division are nonviable.

Plating on caffeine at various times during the division lag showed that the irradiated cells lost almost all of their caffeine sensitivity by the end of the division-delay period, suggesting that repair was completed by that time. This further suggests that caffeine actually is a dark-repair inhibitor in this system and does not serve only as some type of nonspecific metabolic antagonist.

The uncorrected action spectrum for division delay seems to indicate a high degree of protein involvement in the UV effect. The corrected curve more nearly resembled nucleic acid absorption, though shifted to somewhat longer wavelengths. These two action spectra should be considered as the probable extremes. The site of UV action for division delay appears to be of a nucleoprotein nature. Unfortunately, the action spectrum can tell us little more at present. Pomper and Atwood (21), in a review of the action spectra for a number of effects in fungi, found a similar situation for most organisms listed.

The slime mold or bacterial origin of the macromolecules being assayed must be considered before the effect of UV light on their synthesis can be interpreted. The problem is mainly with DNA and results from the fact that slime mold cells use whole bacteria as their sole food source. Thus the possibility exists that some of the DNA being assayed may originate from undigested bacteria contained within the *D. discoideum* cells and not from the slime mold itself. Supplemental experiments pertaining to this question indicated, but did not prove finally, that the DNA assayed was predominantly of slime mold origin. RNA and protein present no such problem, because they are present in such large amounts in the slime mold relative to bacteria.

For the three macromolecular classes tested, synthesis resumed before the period of division delay ended. This is consistent with the microscopic observation of increased cell size during this period. Hanawalt and Setlow (9) found that, in *E. coli*, DNA synthesis was most sensitive to UV light, followed by RNA and protein synthesis to lesser degrees. Slime mold cells appear to overcome the radiation-induced block to DNA synthesis. Setlow et al. (30) showed this to be the case for *E. coli* possessing dark-repair mechanisms but not for those lacking them. Later, Swenson and Setlow (35) were able to correlate the resumption of DNA synthesis with the excision of dimers from the DNA.

Although we have no direct evidence as yet with regard to particular photoproducts or mechanisms, our results strongly suggest that *D. discoideum* vegetative cells are capable of some sort of dark repair of UV damage during the period of division delay and that the repair is concluded at about the time of resumption of division. Photoreactivation under normal circumstances appears to be masked by overlap with this dark repair. UV- and gamma-ray-sensitive strains have been isolated (Deering, *unpublished data*), and these will facilitate the molecular studies needed to further clarify these processes.

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LITERATURE CITED


