*PHOTOREACTIVATION IN AN ESTABLISHED VERTEBRATE CELL LINE**

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Photoreactivation is the amelioration, by exposure to visible light, of biological damage that results from ultraviolet (UV) radiation.[1, 2] The reversal of UV damage is believed, in many cases, to involve the enzymatic monomerization of the cyclobutyl pyrimidine dimers which the UV radiation induces in DNA.[2] The phenomenon of photoreactivation has been observed in a variety of animal and plant phyla[1] although mammalian cells *in vitro* apparently do not photoreactivate UV damage.[3]

This report describes experiments that establish, in a permanent line of marine fish cells, (1) photoreactivation of growth, (2) photoreactivation of DNA synthesis, (3) light-dependent activity that monomerizes pyrimidine dimers *in vivo*, (4) light-dependent activity that monomerizes pyrimidine dimers *in vitro* and also photoreactivates UV-irradiated transforming DNA. Since tissue- and organ-specific functions are generally lost upon prolonged cultivation of cells *in vitro*,[4] it is significant that the phenomenon of photoreactivation is readily demonstrable in a vertebrate cell which has been maintained *in vitro* for over seven years.[5]

Experimental Procedures.—Cell line and media: The Grunt Fin (GF) cell line[5] from fin tissue of the blue-striped grunt, *Haemulon sciurus*, was used in all experiments. GF cells were routinely cultured in grunt fin medium as described by Clem *et al.*[5] GF cells exhibited luxuriant growth at room temperature (21–22°C) in an open system (Petri dishes) without CO_2. The pH of such cultures was 7.8–7.9. During UV and visible light irradiation grunt fin medium was replaced with grunt fin medium lacking phenol red.

Irradiation: GF cells were grown in monolayer in UV-transparent 50-mm-diameter plastic Petri dishes (Falcon Plastics). The cells were irradiated from below with 2650-Å radiation at 5 ergs/mm²/sec from a Hilger monochromator equipped with a 500-watt high-pressure mercury lamp. For photoreactivation treatment (PR) appropriate dishes were illuminated from above for up to 120 min using a standard two-bulb fluorescent desk lamp equipped with one standard 15-watt daylight fluorescent bulb and one General Electric 15-watt "blacklight" bulb (3200–4500 Å) placed at a distance of 30 cm above the Petri dish covers. Glass filters (Corning #7380) which excluded wavelengths shorter than 3500 Å were placed over the cultures during PR. Control cultures were kept in the dark.

Assay of growth and DNA synthesis: Immediately after PR and at 24-hr intervals thereafter the medium was decanted from appropriate dishes, the monolayer was rinsed once in balanced salt solution to remove dead cells, and a 0.02% solution of disodium ethylene diaminetetraacetate (EDTA) in balanced salt solution was placed in the culture dishes. After 15–20 min during which the cells became detached from the dish, the resulting cell suspension was counted in an electronic counter (Coulter Electronics) so as to obtain the total number of live cells per dish.

DNA synthesis was assayed in successive 6-hr periods by adding tritiated thymidine (sp. act. 1.9 c/mM) at a final concentration of 1 μc/ml to the growth medium. After 6 hr the cells were treated as above with EDTA, and an aliquot was counted in the Coulter counter. The remaining cells were then lysed by sonication (Branson Sonifier). The 100-μl aliquots of the sonicate were assayed for radioactivity incorporated into TCA-insoluble material by a modification[6] of the filter disk method of Bollum.[7] At the same time H^3-TdR was added to another set of dishes for the next 6-hr pulse.

Transformation experiments: Photoreactivating activity *in vitro* was measured by the ability

of GF cell extracts to reactivate the biological activity of UV-inactivated transforming DNA. A sonicate of GF cells was prepared in balanced salt solution containing 5.0 mM glutathione and 20% glycerol. (Such preparations could be stored for several months at −20°C without loss of activity.) This whole homogenate was incubated at 37°C in the light or in the dark with transforming DNA from *Hemophilus influenzae* bearing a streptomycin resistance marker. The DNA had previously been UV-irradiated (2442 ergs/mm² at 2537 Å) to ~1% of its initial transforming activity. This DNA was then employed in a transformation experiment with streptomycin-sensitive cells using the procedure of Muhammed.[8]

Analysis of pyrimidine dimers: UV-irradiated *E. coli* DNA containing ~4% pyrimidine dimers was incubated at 37°C in the light or in the dark with a homogenate of GF cells. The DNA was then hydrolyzed with 98% formic acid and analyzed for pyrimidine dimers according to Setlow and Carrier[9] by chromatography on Whatman no. 1 paper with butanol:acetic acid:water (80:12:30, v/v) as the solvent. An additional step was added of second-dimension chromatography in butanol:water (86:14, v/v) to remove background counts from the dimer region of the chromatogram.

The ability of GF cells to monomerize dimers in their own DNA *in vivo* was determined by labeling GF cells with tritiated thymidine for 24 hr, exposing the cells to 150 ergs/mm² of UV at 2650 Å, and then to various doses of PR illumination. Thereafter the cells were collected, precipitated with 5% cold TCA, hydrolyzed, and analyzed for dimers as described above.

Results.—Figure 1 shows the effect of UV doses of 50 and 100 ergs/mm² on the growth of GF cells. Cultures receiving PR illumination after UV exhibited a consistently elevated growth rate compared to those with no PR. By day 4 (a period

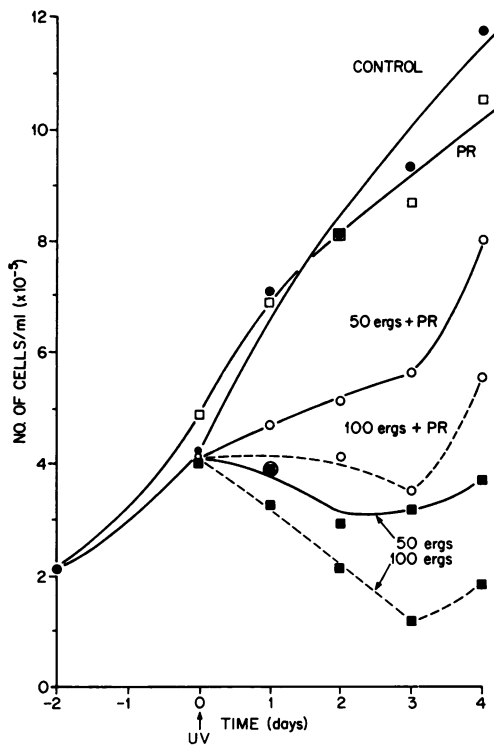

FIG. 1.—Growth of GF cells after irradiation at 2650 Å with either 50 or 100 ergs/mm², with or without subsequent photoreactivation for 120 min (*PR*). Points are means of duplicate plates.

equal to about twice the doubling time of control GF cultures), all the irradiated cultures were apparently resuming logarithmic growth. PR illumination alone had essentially no effect on growth. It is apparent from the data in Figure 1 that PR reduces the effective UV dose by more than a factor of 2.

Periods of PR illumination ranging from 45 to 120 min yielded essentially the same growth recovery in the dose range 50–100 ergs/mm² of UV. At doses of 150 ergs/mm² we found little recovery in the growth of GF cells with or without PR light.

Figure 2 shows the effect of UV and PR illumination on DNA synthesis in GF cells. From 6 to 36 hours after irradiation there was a consistently higher rate of incorporation of label per cell in the cells given UV and PR than in cells receiving UV alone. The decrease in DNA synthesis at 12 hours in all the cultures appears to be due to partial synchrony induced at zero time due to medium change; if the medium is not changed after UV, this decrease is not observed.

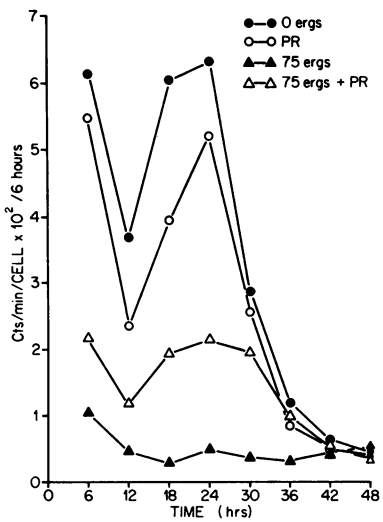

FIG. 2.—Effect of UV (75 ergs/mm² at 2650 Å) and PR on DNA synthesis in GF cells. Cpm H^3-thymidine incorporated per cell during successive 6-hr labeling periods.

The demonstration of photoreactivation in the biological assays suggested that these cells might contain the light-dependent, pyrimidine-dimer-splitting enzyme[2] although indirect photoreactivation[10] was not ruled out. We have detected the activity of this enzyme by assaying for photoreactivation of biological activity of transforming DNA of *Hemophilus influenzae* with GF extracts. In the light, the transforming activity of the UV-irradiated *Hemophilus* DNA was increased by GF cell homogenate to a maximum value 20-fold greater than a corresponding DNA

TABLE 1

PHOTOREACTIVATION *in vitro* BY GF EXTRACT OF UV-IRRADIATED *H. influenzae* DNA*

Material assayed	Assay	Transformants/ml
Irradiated DNA alone	Dark, 60 min	217
Irradiated DNA + GF extract	Dark, 60 min	248
Irradiated DNA + GF extract	Light, 60 min	5239
Irradiated DNA + heated extract (65°C, 10 min)	Light, 60 min	465

Transforming DNA: 0.25 μg/ml (irradiated to ~1% survival with 2442 ergs/mm² at 2537 Å).
Protecting DNA (calf thymus): 80 μg/ml (to protect transforming DNA from GF nucleases).
Extract: 1 mg/ml protein.
Light: Blacklight, 8000 ergs/mm²-min.
Temperature: 37°C.

* Assayed as transforming activity of streptomycin-resistancemarker with streptomycin-sensitive cells.

sample incubated under the same conditions in the dark (Table 1). Under the conditions of this assay, the 20-fold increase represents repair of 90 per cent of the UV damage in the transforming DNA, and is the maximum extent to which such DNA can be repaired by the more fully characterized photoreactivating enzyme from baker's yeast.[8] Heating the GF homogenate to 65°C for 10 minutes drastically reduced the photoreactivating activity.

The repair of 90 per cent of the UV-induced lesions in transforming DNA as assayed in the transformation system was strong evidence that pyrimidine dimers in the DNA had been reversed.[2] We examined this point directly by using chromatographic techniques to measure the disappearance of pyrimidine dimers in UV-irradiated *E. coli* DNA exposed to GF cell homogenates in the light or in the dark as in the previous experiment. The *E. coli* DNA was irradiated with a different dose and at a different wavelength from the *Hemophilus influenzae* DNA and therefore the results are not quantitatively comparable. The thymine–thymine ($\widehat{T–T}$) homodimers and the uracil-thymine ($\widehat{U–T}$) heterodimers (which arise from the deamination of cytosine-thymine ($\widehat{C–T}$) heterodimers during hydrolysis of the DNA) were distinguishable in the chromatographic system used. Figure 3 shows

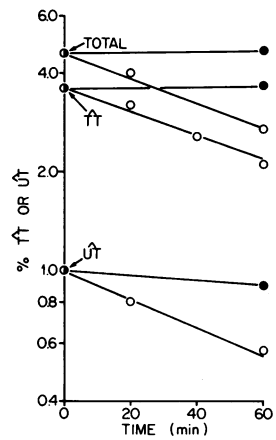

FIG. 3.—Light-dependent dimer reversal of dimers in *E. coli* DNA by GF cell homogenate; 0.25 μg *E. coli* DNA (H^3-thymidine labeled) irradiated with 10^4 ergs/mm² at 2804 Å was incubated with GF cell homogenate at a final concentration of 0.5 mg protein/ml. *Ordinate:* per cent of total thymine as thymine-thymine or uracil-thymine dimer. *Abscissa:* time of incubation at 37°C. *Closed circles:* dark. *Open circles:* blacklight, 8000 ergs/mm²-sec.

the disappearance of the two kinds of dimers from the TCA-insoluble material during exposure of the DNA to the GF cell homogenate in the light. Both kinds of dimers were lost at approximately the same rate. There was essentially no loss of dimers during incubation of irradiated DNA with GF extract in the dark. The TCA-soluble fraction contained no measurable amount of dimers after 120 minutes PR.

Finally it remained to show that, in the light, UV-induced pyrimidine dimers in GF–DNA were also eliminated in irradiated, intact GF cells. For this purpose a low UV dose (150 ergs/mm²), yet one near the limit which GF cell populations can survive, was used. At such low doses the number of thymine-containing dimers formed is small, about 0.3 per cent of the total thymine, and the accuracy of the measurements is correspondingly reduced. Nevertheless, the reduction in number of both $\widehat{T–T}$ and $\widehat{U–T}$ dimers during a two-hour period of PR illumination is clearly measurable (Fig. 4). Again, the rates of loss of the two types of dimer are of the same order of magnitude, and again the lost dimers are not detected in the TCA-

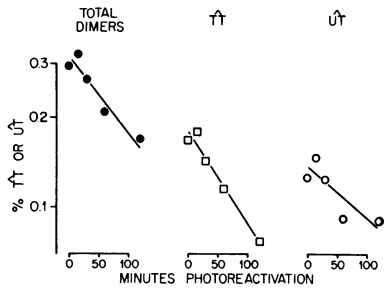

FIG. 4.—Light-dependent dimer reversal *in vivo* in GF cells which had received 150 ergs/mm² of 2650 Å UV. *Ordinate:* percent of total thymine as thymine-thymine or uracil-thymine dimer. *Abscissa:* time in photoreactivating light at 25°C.

soluble component of the cells. These observations are consistent with the hypothesis that the dimers are monomerized rather than excised.[11] During the two-hour incubation there was no detectable loss of dimers from the UV-irradiated controls kept in the dark.

Discussion.—These studies are the first demonstration of light-dependent repair of UV damage to vertebrate cells in culture. In fact, *Paramecium aurelia* is the only other eukaryotic cell which has previously been shown to have a light-dependent mechanism for the monomerization of pyrimidine dimers *in vivo*.[12]

The only known reaction forwarded by the photoreactivating enzyme is the monomerization of pyrimidine dimers.[2, 11] We have shown here an activity of this kind in GF-cell extracts, and hence it is reasonable to suppose that the biological PR which we observe in these cells is due to the reversal of pyrimidine dimers. However, growth experiments have shown essentially no difference in recovery of GF cells receiving either 45 or 120 minutes of PR after either 50 or 100 ergs/mm² of UV, whereas dimers continue to be lost from GF–DNA *in vivo* throughout the 120-minute PR period. This seems to indicate that not all pyrimidine dimers are equally significant in cell survival, although nothing is known of the actual mechanism whereby pyrimidine dimers affect survival in eukaryotic cells.

Cleaver[3] examined several mammalian cell lines for photoreactivation and found none. Chinese hamster cells show no elimination of UV-induced pyrimidine dimers with subsequent visible-light irradiation.[13] Cook and McGrath[14] found no photoreactivating enzyme in a variety of mammalian tissues. Present information thus seems to indicate that mammalian cells *in vitro* possess no light-dependent repair mechanism. There is, however, some evidence for the possible existence of an excision-type dark repair mechanism in human cells[15] but not in Chinese hamster or mouse cells[16] *in vitro*. Chick embryo cells can photoreactivate UV-irradiated pseudorabies virus[17] and contain photoreactivating enzyme, but most adult chicken tissues lack such enzyme activity.[14]

Amphibian tissues are replete with photoreactivating enzyme[14] and we have evidence for biological photoreactivation in an established cell line from amphibian liver.[18]

The present observations are particularly significant in that GF cells have been cultured *in vitro* for over seven years.[5] Since tissue-specific functions are usually lost upon prolonged *in vitro* cultivation of cells,[4] photoreactivation can be considered a fundamental cellular property which is probably common to all cells of the donor organism and by extension is thus quite likely a characteristic of fish cells in general. GF cells present the first case in which photoreactivable biological re-

covery and molecular repair can simultaneously be studied in an established cell line. GF cells should present a valuable tool in the study of DNA repair mechanisms in the cells of higher organisms.

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