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TRANSIENT INDUCTION OF PHOTOLYASE ACTIVITY IN ARRESTED FROG CELLS IN RESPONSE TO A SHORT-WAVE ULTRAVIOLET SEGMENT OF SIMULATED "SUNLIGHT"*

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SUMMARY: Induction of photolyase activity was studied in cultured frog cells using clonogenic assays. Exposure of arrested cells to a pre-irradiation (90% survival) of 254nm ultraviolet light resulted in a transient enhancement of photolyase activity. Cells expressed a decreased level of photolyase activity in response to an equitoxic fluence of simulated "sunlight" wavelengths 280-310nm. However, no significant increase of enzyme activity was detected in cells following treatment with "sunlight" wavelengths 310-330nm. In addition, this process depends on newly biosynthesized protein(s).

The existence of an inducible DNA repair pathway, termed SOS function, has been clearly demonstrated in E. coli [1, 2]. Similar phenomenum has also been speculated to occur in yeast [3, 4, 5] and insect cells [6]. In higher eukaryotes, however, the existence of such an inducible system has remained a contraversial issue [7, 8, 9]. Evidence accumulated in recent years strongly suggests that the repair system in higher eukaryotes is regulated. This data includes i) an enhanced survival and mutation of viruses in ultraviolet (UV)-stimulated mammalian host cells [9], ii) the involvement of proteases in human cells responsive to DNA damages [10, 11], iii) the induction of specific

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Abbreviations: EPR, enzymatic photorepair; PBS, phosphate-buffered saline; PRL, photoreactivating light; PR sector, photoreactivable sector; SLM, Mylar-filtered sunlamp; UV, ultraviolet.

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nascent protein(s) in human cells following exposure to UV light [12, 13, 14], and iv) excision repair can be dually or multiply regulated in Drosophilla [16, 17] and yeast [4, 5, 18]. In addition, UV light activates a quiescent mouse gene for metallothionein-I possibly resulting from UV-induced changes of the DNA methylation pattern [15]. Without further data, it is speculated that the observed protease activity and UV-induced nascent protein(s) in mammalian cells in response to UV damages may be comparable to those found in the E. coli SOS function.

We have been interested in the light-dependent enzymatic (photolyase) repair in higher eukaryotic cells. This system specifically repairs UV-damaged DNA by direct reversal (i.e., monomerization) of cyclobutane-type pyrimidine dimers [9]. It has been shown that amphibian cells are abundant in photolyase activity, but poorly express dark repair enzymes [19]. Taking advantage of this simplified repair system, we attempted to determine whether enzymatic photorepair (EPR) is inducible in higher eukaryotic cells, and whether the induction of this repair activity can be durally regulated or if it responds only to its substrates, i.e., cyclobutane pyrimidine dimers. Using clonogenic assays, we compared the induction of photolyase activity in cultured ICR 2A frog cells by different segments of simulated "sunlight" wavelengths reaching the earth's surface which cause different types of DNA damages [20]. We found that 1) the photolyase activity can be transiently induced in this eukaryotic cell line; 2) the induction of enzyme activity in cells is unique to cyclobutane-type dimer DNA damage; and 3) the induction is dependent on newly synthesized protein(s).

MATERIALS AND METHODS

Cell lines and arrested cultures. Cultures of ICR 2A frog cells derived from the frog Rana pipiens were maintained as described [21]. These growing cells have a population doubling time of 48 h. Arrested (non-dividing) cultures were established by serum deprivation and maintained by a modification of published procedures [22, 23]. In brief,

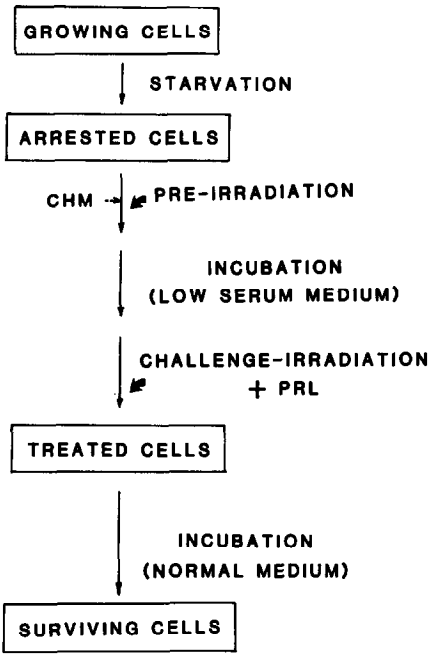

Figure 1. Schematic representation of the experimental strategy. The surviving fraction of cells was used to calculate EPR induction. See materials and methods for detail. CHM, 2 μM cycloheximide included in some culture medium.

cells were plated into 60 mm tissue culture dishes (Corning Glass Works, Corning, NY) at different densities representing three logrithms for cellular clonogenic assays. After 24 h incubation in medium supplemented with 10% serum, the medium was then changed for one containing low (0.5%) serum and incubated for 5 days. This was day 0 of the experiments. During the period of the stabilization in low serum, the population had slightly increased and then reached a healthy equilibrium level without further cell division.

Irradiation conditions and clonogenic assays. Arrested cells were washed twice with phosphate-buffered saline (PBS) and irradiated in PBS as previously described [24]. The irradiation source was either germicidal lamps (254nm) or simulated "sunlight" segments generated by sunlamps filtered through Mylar 8C [SLM(8C)] or through Mylar 48A [SLM(48A)]. SLM(8C) and SLM(48A) represent wavelengths 290-310nm and 310-330nm respectively [20, 25]. Following irradiation or mock treatments, cells were washed, incubated for six gerneration doublings (12 days), fixed and then stained with Giemsa. The number of surviving colonies (groups of 32 or more cells) was determined by inspection with a stereo microscope. Extended growth curves were performed by visual observation of 25 microscopic "windows" of the same culture every other day using the inverted microscope.

Photolyase activity assays. The strategy of the clonogenic assay of photolyase activity is schematically represented in Fig. 1. Arrested cells which had received a pre-irradiation were incubated for a period of time, washed with PBS and either sham irradiated or exposed to a challenge fluence of 254nm-UV immediately followed by a fluence of photoreactivating light (PRL) [26, 27]. A nontoxic concentration of antibiotic cycloheximide (2μM), a protein synthesis inhibitor, is included

in some culture dishes during and after pre-irradiation incubation in low-serum medium. Cultures were then washed again with PBS, incubated in normal medium, and the clonogenic ability was determined. Since unrepaired cyclobutane dimer DNA damages can account for the UV cytotoxicity [28] and the EPR of such damage is an unique function of photolyase [9, 29, 30], the relative activity of photolyase can therefore be estimated by the enhancement of cellular clonogenic efficiency. The induction index was calculated from the optimal survival fraction of cells with pre-irradiation divided by that without pre-irradiation for each case [31].

RESULTS AND DISCUSSION

Typical survival curves of ICR 2A frog cells following UV-irradiation are shown in Figure 2. Photoreactivable sector (PR sector), an indicator of the functional role of cyclobutane dimers, were calculated according Jagger [32]. The PR sector for 254nm-UV, for example, is 0.85 which means 85% of cell death was caused by cyclobutane dimers. As shown in each panel, the order of PR sectors is

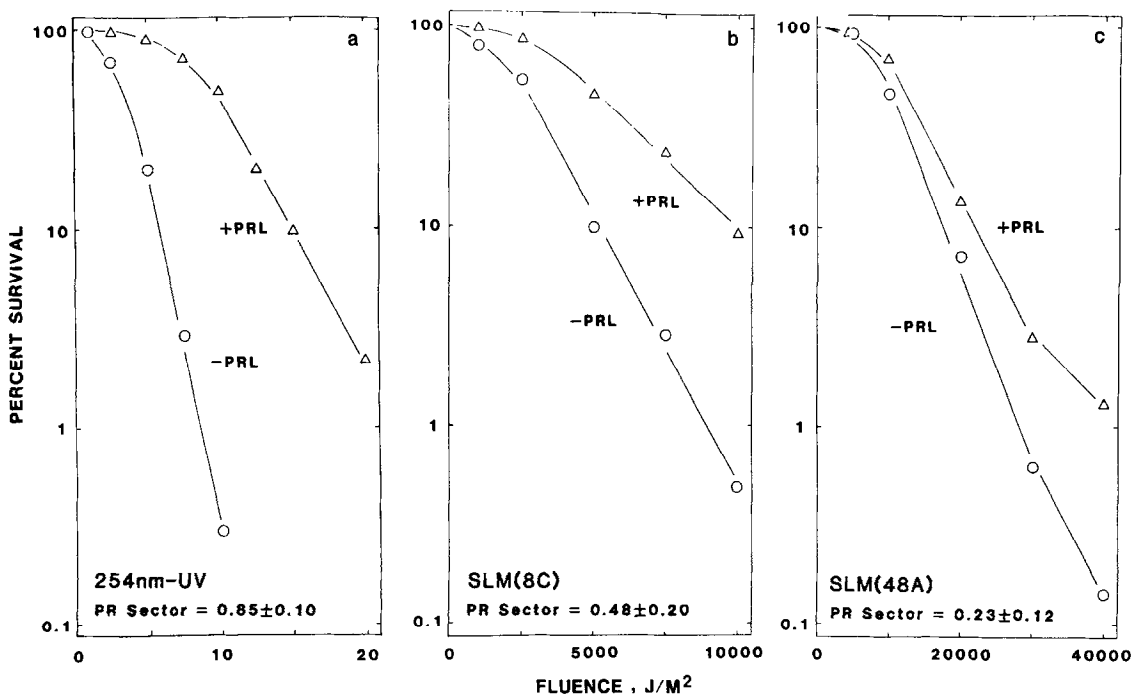

Figure 2. Clonogenic ability of cells following UV-irradiation. Cells were exposed to UV-irradiation as indicated in each panel, and then either immediately followed by a fluence of PRL (+PRL) or kept in the dark (-PRL). The PR sector (with standard error) for each treatment is indicated. Each point represents an average of triplicate samples.

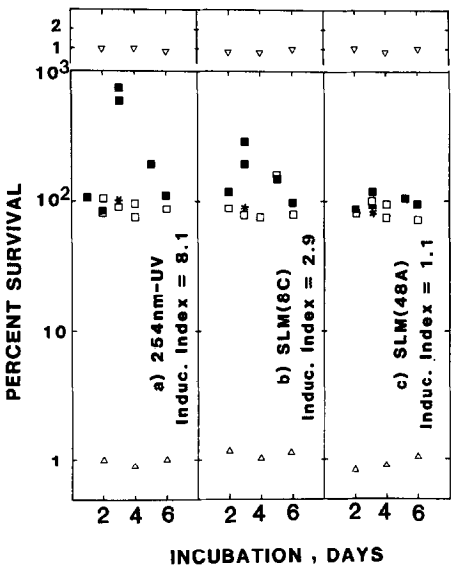

Figure 3. Induction of EPR in arrested cells. Cells without (□) or with (■) pre-irradiation as shown in each panel were treated with a challenge fluence plus PRL, and the clonogenic abitily was determined. Response of cells pre-irradiated and exposed to a challenge fluence alone is presented by the symbols (△). The EPR induction for samples incubating in medium containing 2 μM cycloheximide is also shown (✱). The average induction index for each case is indicated. The top panel represents the relative cell number, refered to the cell number at day 0, during the course of incubation.

254nm > SLM(8C) > SLM(48A), indicating that non-dimer DNA damages play an increasingly functional role in cells exposed to a segment of increasing UV wavelengths. A similar conclusion was previously made using mammalian systems [33]. Hence, the action of SLM-UV on cells is different from the action of 254nm-UV light.

Induction of photolyase activity in arrested cells by different qualities of radiation was measured using clonogenic assays. Plots of typical surviving fraction against time after an inducing treatment (i.e., pre-irradiation) are shown in Figure 3. Cells treated with 254nm UV showed the greatest EPR induction at day 3 after pre-irradiation before declining back to the baseline level (Fig. 3a). Such a pattern is less drastic, but is stil seen for cells exposed to SLM(8C) (Fig. 3b). However, it was not possible to generate a similar curve for cells

exposed to SLM(48A) (Fig. 3c). The slight induction seen in SLM(8C)-treated cells probably is due to a response to a small portion of dimer damages also induced in cells [20, 25, 34, 35]. This interpretation was confirmed by the observation that PRL treatment immediately after pre-irradiation to remove cyclobutane dimers eliminated the photolyase induction (unpublished results). The induction index for each case is calculated. As indicated in the figure, cells exposed to 254nm-UV expressed an eight-fold enhancement of EPR as opposed to a three-fold increase by SLM(8C). Cells which received a pre-irradiation of SLM(48A) expressed a baseline level of enzyme activity (induction index = 1.1). EPR induction in cells pre-treated with r-ray, a known DNA-damaging agent which causes a relatively pure population of single strand breaks (SSBs) were also tested. No induction was observed for the latter case (unpublished results). Since SLM(48A), like r-ray, causes a large amount of SSBs as opposed to 254nm [20], and the order of EPR induction is 254nm > SLM(8C) > SLM(48A), the induction of photolyase activity in damaged cells probably is not due to SSBs and rather caused by cyclobutane dimers. Neverthless, we still could not explain why the cyclobutane dimers induced by SLM(48A) in cells, although at a relatively small amount (as reflected from the PR sector), were unable to elicit EPR induction. For each case, addition of nontoxic cycloheximide to the incubation mixture completely blocked the EPR induction (shown as * symbol for each treatment in Fig. 3), suggesting that the induced photolyase activity in cells depends upon newly synthesized protein(s). It has been shown that excision repair in yeast cells can respond both to 254nm-UV damages and heat shock [5, 17] or chemicals [4, 5, 18]. It will be interesting to see whether there is a similar dually regulated mechanism present in frog cells. It will be very surprising if EPR is regulated by non-dimer type DNA damages because it is difficult to understand why it is necessary for cells to respond to DNA damages by synthesizing protein(s) which will not be utilized.

Studies of the induction of photolyase activity in the same cell line by stresses other than radiation are currently being undertaken.

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