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Time Course of PR of UV-Induced Chromosomal Aberrations and Lethal Damage in G1 Xenopus Cells

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ABSTRACT

Synchronous cultures of early G1 cells were exposed to UV and their ability to photoreactivate lethal and aberrational damage was determined as a function of time following UV exposure. Lesions leading to cell death were converted to a non-photoreactivable state before cells entered S phase, while lesions leading to chromosomal aberrations were converted to a non-photoreactivable state as the cells entered S phase. These results indicate that the intracellular mechanism which expresses photoreactivable UV-induced lesions in G1 cells as cell death is not identical to the mechanism which expresses such lesions as chromosomal aberrations, and the two mechanisms operate primarily in different phases of the cell cycle.

INTRODUCTION

A number of vigorously growing non-mammalian vertebrate tissue culture cell lines have now been established which possess repair mechanisms that promise to add useful parameters to study of cellular radiation response. The A8W243 *Xenopus* line has an unusually efficient photoreactivation (PR) mechanism for repair of ultraviolet (UV)-induced lesions leading to chromosomal aberrations and cell death (Griggs and Bender 1972, 1973). It is believed that such UV-induced primary lesions are pyrimidine dimers in DNA, since these dimers are the only UV-induced lesions that have been shown to be subject to PR (Smith and Hanawalt 1969). This paper deals with an initial study designed to describe the manner in which UV-induced primary lesions and associated aberrant intracellular mechanisms in early G1 A8W243 cells that produce cell death are related to mechanisms producing chromosomal aberrations. Study of PR of cell killing and aberration production as a function of time following UV exposure should aid in describing the degree of overlap of primary lesions and associated intracellular mechanisms by revealing "when" following irradiation and "where" in the cell cycle the UV-induced damage is converted to a non-photoreactivable state.

MATERIALS AND METHODS

Most of the techniques employed with the A8W243 *Xenopus* line such as routine maintainence of log phase cultures, culture incubations, methods of obtaining synchronous cultures, and irradiation procedures have been described in detail by Griggs and Bender (1972) and Orr and Griggs (1976).

Cell survival assays and chromosomal aberration analysis were carried out by one observer using standard techniques (Griggs and Bender 1972, Wolff 1961).

RESULTS AND DISCUSSION

Results of a study dealing with the time course of PR of UV-induced lethal damage is shown in Figure 1. The curve clearly indicates that PR of this damage is significantly diminished shortly after UV exposure, becoming negligible by the 10 hr point. The intracellular mechanism which converts the primary lesions to non-photoreactivable lethal lesions begins to operate shortly after UV exposures.

Figure 2 shows results of a flash labeling experiment to determine the manner in which irradiated and non-irradiated early G1 cells progress through G1 and enter S phase. Both irradiated and non-irradiated cells move through G1 and enter S in essentially the same manner, and the data clearly shows the length of the G1 phase to be around 8 hrs. These results coupled with the results of Figure 1 indicate that many of the UV-induced photoreactivable lesions which lead to cell death are converted to a non-photoreactivable state before the cells enter S phase, and the associated intracellular mechanism must be operating in G1.

The time course of PR of aberrations was carried out as follows. Two series of synchronous cultures of early G1 cells were prepared. Series 1 was used for mitotic index determinations, because preliminary experiments had shown that the interaction of the PR mechanism and the intracellular mechanism by which some UV-induced damage is expressed as division delay significantly alters the manner in which irradiated G1 cells progress through S to mitosis. Thus, detailed mitotic index data were required in order to properly describe peaks of mitotic activity following various treatments and facilitate collection of appropriate samples for aberration analysis. Series 2 was used for chromosome analysis. All cultures were exposed to UV (120 ergs mm^{-2}) one hr after mitotic selection and, at the times indicated in Table 1, white light (3×10^{-3} ergs mm^{-2}) was administered to subsets of each series. Some of the desired mitotic index data is shown in Figure 3. The data of Table 1 clearly indicates that most of the UV-induced damage leading to aberrations can be photoreactivated at points throughout the G1 period (0.25 - 6.0 points), but as exposed cells enter S phase (points 7 & 8) the UV-induced primary lesions are converted to a non-photoreactrivable state. Since chromosome-type aberration frequencies observed did not exceed controls, only chromatid-type aberration frequencies were recorded in Table 1.

Bender, Griggs and Walker (1973) have proposed a model for UV-induced aberration production based on the notion that the eukaryotic chromosome contains a single DNA double helix, and that a mechanism, similar to the repair mechanism described by Fujiwara (1972), plays an integral part in the conversion of pyrimidine dimers to chromatid-type aberrations. The mechanism functions during S phase. When DNA replication reaches a dimer, a "gap" is left in the newly synthesized strand. This "gap" is often not repaired and leads to an aberration. Data from Table 1 support the model by indicating that the mechanism which converts dimers in DNA to a non-photoreactivable state begins to operate in early S. Furthermore, as indicated by the 7, 8, and 30 PR points, the time interval during which the mechanism seems to operate at optimum efficiency coincides with the interval of maximum DNA synthesis.

In summary, the intracellular mechanism which expresses photoreactivable UV-induced lesions in G1 cells as cell death is not identical to the mechanism which expresses such lesions as chromosomal aberrations, and the two mechanisms operate in different phases of the cell cycle.

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Table 1. Time course of PR of UV-induced chromatid aberrations in G1 cells

PR Time (hours after UV)	Cells scored	Aberrations deletions	Aberrations exchanges
0.25	400	7	4
1.25	"	7	6
2.00	"	9	6
4.00	"	10	5
6.00	"	8	6
7.00	"	219	197
8.00	"	396	421
30.00	"	410	404

Figure 2. Labeling curves to describe how non-irradiated (circles) and irradiated (triangles) early G1 cells progress through G1 and enter S phase. Irradiated cells were exposed to 120 ergs mm^{-2} UV one her after mitotic selection.

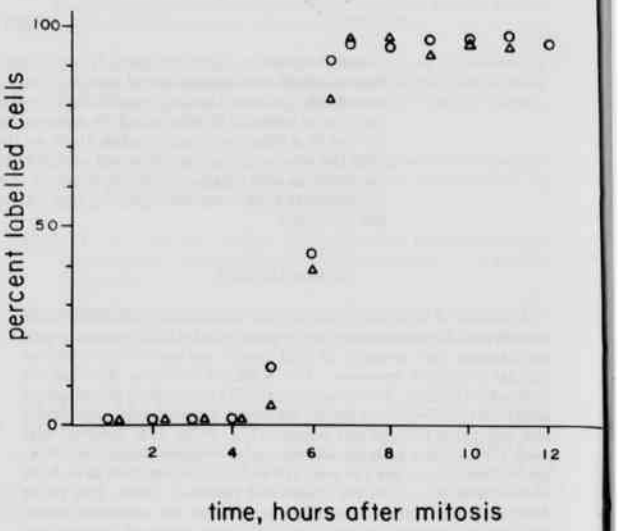

Figure 1. Time course of PR of UV-induced lethal damage in early G1 cells. 10^3 cells studied at each dose point. Cultures were exposed to 120 ergs mm^{-2} UV 90 minutes after mitotic selection and 3 x 10^4 ergs mm^{-2} white light at the indicated times following UV exposures.

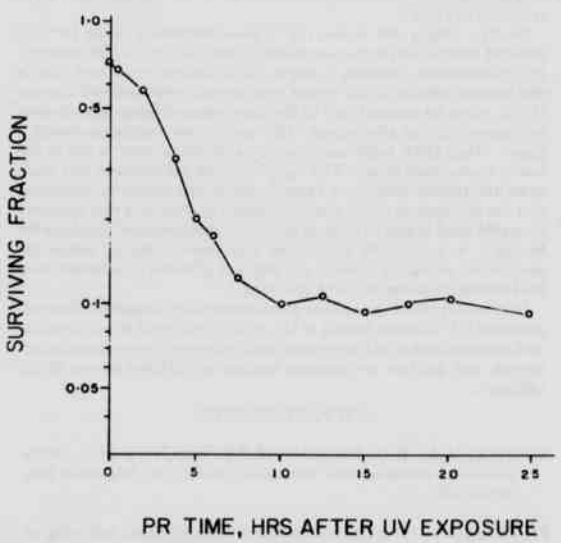

Figure 3. The manner in which early G1 cells progress through interphase and the first mitosis after being exposed to 120 ergs mm^{-2} UV (1 hr after mitosis) and 3 x 10^4 ergs mm^{-2} white light. A: 0.25 hr after UV. B: 4 hr after UV. C: 6 hr after UV. D: 8 hr after UV.