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PHOTOREACTIVATION OF UV-INDUCED DAMAGE IN G1 PHASE XENOPUS CELLS THAT LEADS TO SISTER CHROMATID EXCHANGED AND CELL DEATH

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ABSTRACT

Experiments were conducted with the A87 *Xenopus* tissue culture cell line which centered on use of the line's efficient photoreactivation (PR) mechanism to: (1) determine the extent to which sister chromatid exchanges (SCEs), induced by exposing early G1 phase cells to low UV fluenced, are photoreactivable, and (2) determine the extent to which the photoreactivable SCEs resulting from these low UV fluences constitute lethal lesions. For the first determination, UV fluences – SCE frequency relations and UV fluence + PR fluence – SCE frequency relations were established for UV fluences in the range 0 - 12 J/m^2 and a single PR fluence of 22,000 J/m^2. Comparison of these relations indicated that the cells photoreactivated a predominant fraction (near .70) of the induced SCEs. For the second determination, a detailed time course of PR of induced SCEs relation and a time course of PR of induced lethality relation were established for the cells, using a single UV fluence of 5.0 J/m^2 and a single PR fluence of 22,000 J/M^2. Comparison of these relations indicated that few, if any, photoreactivable SCEs constituted photoreactivable lethal lesions. This comparison also suggested that further high resolution cytological studies of time course of PR of UV-induced SCEs may reveal additional relations between repair of SCEs and changes in vertebrate chromosome structure as cells progress through interphase.

INTRODUCTION

Sister chromatid exchanges (SCEs) result from interchanges between DNA molecules at homologous loci within replicating chromosomes. The biological significance of SCE production is not known. Knowledge of the mechanisms of SCE production are incomplete and present interest in their exploration derives, in part, from the notion that understanding of these mechanisms will assist in identifying the biological significance of SCEs. Interest in the exploration of these mechanisms also stems from results of previous studies (Latt, 1981) indicating numerous parallels between SCE induction and mutagenesis, including the fact that many agents that are efficient at producing SCEs are also highly mutagenic. These parallels strongly suggest that studies of SCEinduction might yield useful results that could be extrapolated to mutagenesis and vice versa. Since a bromodeoxyuridine (BrdU0-Giemsa dye differential staining technique is now available for assaying SCEs, that is much more efficient and easily applied than available techniques for assaying mutations (Perry and Wolff, 1974), studies of the mechanisms of SCE induction are receiving increasing emphasis. There studies are designed primarily to describe the lesions induced in DNA that lead to SCEs and intracellular processes which express them.

Since shortwave UV is exceptionally effective at producing both SCEs and mutations, considerable attention has been focused on attempts to describe the primary DNA lesions induced by this agent that lead to SCEs and related expression mechanisms. The observation that the major primary lesions induced in DNA by UV are pyrimidine dimers (Sutherland, 1981) led a number of investigators to perform experiments designed specifically to relate pyrimidine dimer induction to SCE induction. The general approach used in these experiments was to determine the extent of photoreactivation (PR) of UV-induced SCEs in PR competent cells. This approach was based on the previous observation (Sutherland, 1981) that, apparently, pyrimidine dimers are the only photoreactivable DNA lesions, implying that SCEs could be photoreactivated in PR competent cells "only" if pyrimidine dimers are lesions that lead to SCEs. Kato (1974) reported successful attempts to photoreactivate UV-induced SCE production in rat kangaroo *(Potorous)* cells. However, Wolff (1978) was unable to repeat Kato's (1974) experiments. More recently, Ishizaki *et al.* (1980) made another attempt to photoreactivate UV-induced SCEs in *Photorous* cells and obtained results which were more consistent with Kato's observations. Wolff (1978) also failed to observe PR of SCEs induced by UV in chick embryonic cells, although he did detect chemical evidence of a significant level of PR of the induced pyrimidine dimers in these cells. In contrast, Natarajan *et al.* (1980) reported a successful attempt to photoreactivate significant levels of both pyrimidine dimers and SCEs, induced by UV in chick embryonic cells. Both *Potorous* cells and chick embryonic cells possess negative properties that detract from their suitability as good materials for studies of PR of SCE formation; the chick cells have a very difficult karyotype for SCE and other chromosome analysis (Natarajan *et. al.* 1980), while *Potorous* cells possess a relatively inefficient PR mechanism (Wolff, 1978). These facts coupled with observations by Little (1978), that SCE induction by some agents is very sensitive to experimental protocol, suggest that differences (which might normally appear minor) in experimental protocols and accompanying data analysis used by Wolff and the other investigators might account, at least in part, for the contradictory observations. The A8W243 *Xenopus* lines derived from the A8W243 line, do not possess the negative properties possessed by the *Potorous* and chick cells. Instead, *Xenopus* cells possess very stable karyotypes, consisting of relatively large metacentric and submetacentric chromosomes that constitute quite suitable material for SCE analysis. Furthermore, these cells possess mechanisms for PR of UV-induced lethal damage and chromosomal abberrations not surpassed (to our knowledge) by any other cell line. Therefore, it appeared to us that these cell lines would constitute quite suitable material for PR studies which might assist in resolving the apparent conflict in the data mentioned above, and, perhaps, otherwise lead to significant additions to our knowledge of SCE induction by UV and its biological significances. We describe here the detail of our first experimentation in this direction, which was primarily designed to enhance knowledge of the role of UV-induced photoreactivable pyrimidine dimers (PPds) in UV-induced SCE production and the role of UV-induced photoreactivable SCEs (PSCEs) in UV-induced cell-killing.

MATERIALS AND METHODS

All experimentation was performed with the A87 *Xenopus* cell line, which was recently cloned from the A8W243 line described by Griggs and Bender (1972). Monolayers of A87 cells were routinely maintained in the dark at 22°C in large plastic bottles (Falcon) in F10 medium (Gibco), supplemented with 10 percent foetal calf serum (Hazleton) and buffered with HEPES (Sigma).

In exponential growth at 22°C, the cells exhibited a plating efficiency near 92%, and an average cycle time of 38 hours (12 hours G1; 18 hours S; 5.5 hours G2; 2.5 hours M). The line has a relatively stable karyotype with virtually 96 percent of the cells possession 36 easily identifiable chromosomes (Figure 1a) and constitutes relatively good cytological material for various chromosomes analyses, including SCE detection.

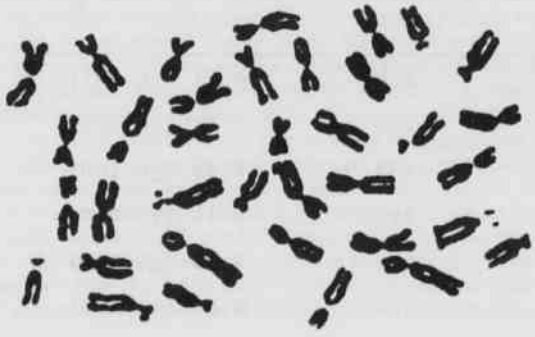

Figure 1a. Photograph of a normal set of A87 *Xenopus* chromosomes.

Techniques employed for single cell plating, colony assays, survival curve analysis, cell synchronizations, mitotic index determinations, mitotic arrest, and preparation of chromosome spreads did not differ significantly from those described in detail previously (Griggs and Bender, 1972; Griggs and Orr, 1979; Griggs and Payne, 1981; Kulp and Griggs, 1989).

All UVand PR irradiations were carried out with the same apparatus as described by Griggs and Orr (1979). UV was administered under red light at a fluence of 5 J/m²/sec at 22°C. The PR scheme used was the same as the one determined by Kulp and Griggs (1989) to be optimum for PR of UV-induced aberrations in the closely related A86 *Xenopus* line. A preliminary experiment to determine how the cells progress through G1 phase into S phase was carried out with synchronous cell cultures following 5 J/m² UV fluence, 5 J/m² UV fluence + 22,000 J/m² PR fluence, and a control with no UV fluence or PR fluence. The results are shown in Figure 2.

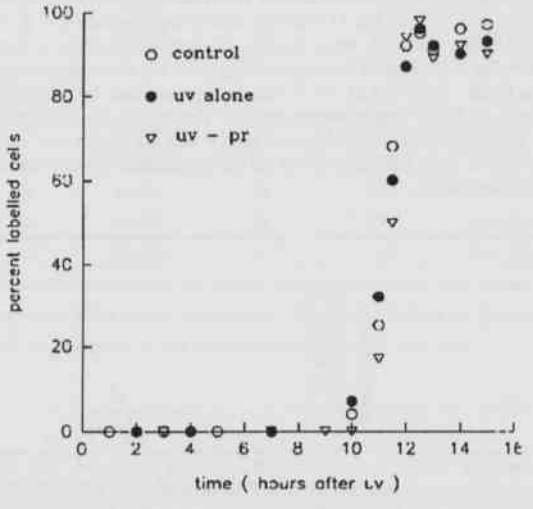

Figure 2. Time course curve to determine how the cells progress through G1 phase and into S phase following no fluences (open circles), 5 J/m² UV fluence (filled circles), and 5 J/m² UV fluence + 22,000 J/m² PR fluence (triangles).

Synchronous *Xenopus* cell culture were used in the experimentation for two reasons: (1) Analysis of effects induced by UV and related repair in non-synchronous cultures is far more complex than in synchronous cultures because cells in different phases of the cell cycle exhibit significantly different radiation sensitivities and repair potentials. (2) The SCE detection technique used essentially precluded use of non-synchronous cultures.

A 5-bromodeoxyuridine (BrdU) labelling-Giemsa differential staining method, similar to that developed by Perry and Wolff (1974), was used for detecting SCEs produced by UV and/or PR treatments of synchronous monolayers of G1 cells and their progeny. Monolayers of cells, synchronized in early G1 phase, were allowed to progress through S phase in medium containing BrdU to a final concentration of 5 x 10^{-4} molar or 0.0 molar. Shortly after the passage of the cells through S phase, the medium was removed and replaced with non-BrdU medium. Mitotic selection at this first mitosis produced cultures of unifiliarly labelled cells or non-labelled cells that were then allowed to progress through G1 and early S phase in non-BrdU medium to the second mitosis. As the cells progressed through the second G1 and early S phases, they were exposed to the desired fluences of UV and/or PR light. When the treated cells reached the second mitosis, samples of metaphase cells were collected with colcemid (Sigma). Metaphase spreads prepared from these samples on microscope slides were stained by the following procedures in the order listed: (1) The slides were completely covered with drops of Hoechst 33258 solution (.150 mg/ml) in Sorensen's buffer, let stand in the dark for 25 minutes, and then rinsed thoroughly in distilled water. (2) Slides were mounted with 2 x SSC (0.3 M NaCl, 0.03 M sodium citrate) and exposed to a bank of ultraviolet lights (Westinghouse F20) for 2 hours. (3) Coverslips were carefully removed from the slides and, after a thorough rinse in distilled water, each slide was stained for 5 minutes in freshly prepared Giemsa solution (5 percent Gurr's R66 in Sorensen's buffer, pH 7.0). This differential method produces faint staining of chromatids which contain unifiliarly BrdU-substituted DNAand bright staining of chromatids which contain no BrdU (Figure 1b).

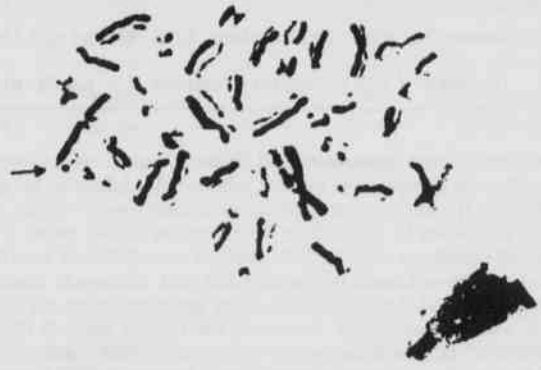

Figure 1b. Photograph of a set of A87 *Xenopus* chromosomes following the BrdU labelling-Giemsa differential staining method and showing a sister chromatid exchange (arrow).

RESULTS AND DISCUSSION

Table 1 contains data from the initial set of experiments performed. These experiments were designed to obtain an indication of the extent of PR of UV-induced SCEs in A87 cells. The starting point in each of these experiments was UV irradiation of a large set of synchronous cultures of early G1 phase cells, that were unifiliarly labelled with BrdU. Cultures of cells in early G1 phase were used because cultures obtained by mitotic selection possess their best synchrony while in early G1 phase, and the complexity of analysis of effects induced in *Xenopus* cultures by UV is significantly greater in cultures with diminished synchrony. The set of UV exposed cultures was then subdivided into four subsets (A,B,C,D).

Photoreactivation of UV-Induced Damage in G1 Phase Xenopus Cells

Table 1. PR of lesions, induced in early G1 phase A87 cells by UV, that lead to SCEs.

Experiment number*	UV fluence (J/m^2)	PR fluence (J/m^2)	Time range for cell collection by colcemid (hrs after UV)	Number of cells scored	Number SCEs per cell (± standard error)
1	0	0	34 - 45	250	0.01 (±0.001)
2	0	22,000	34 - 45	250	0.07 (±0.001)
3	1	0	40 - 58	250	1.45 (±0.100)
4	1	22,000	36 - 54	250	0.32 (±0.010)
5	2	0	45 - 60	250	2.50 (±0.150)
6	2	22,000	38 - 50	250	0.80 (±0.030)
7	5	0	72 - 100	250	6.80 (±1.120)
8	5	22,000	55 - 80	250	1.40 (±0.160)
9	8	0	80 - 105	250	7.90 (±1.390)
10	8	22,000	65 - 90	250	2.00 (±0.180)
11	12	0	90 - 120	250	9.10 (±1.680)
12	12	22,000	75 - 100	250	2.80 (±0.210)

* PR was administered to synchronous cultures (unifiliarly labelled with BrdU) immediately following the termination of UV. Two samples of mitotic cells, equal in number (125), were scored in each experiment.

Subsets A and B were photoreactivated. Subsets A and C were allowed to progress through interphase to the first mitosis (M1) following the exposures. Subsets B and D were used for detailed mitotic index studies, some of which are shown in Figure 3, to determine the mitotic peaks at M1. Samples of metaphase cells for SCE analysis were collected from these mitotic peaks by colcemid treatments. The data of the odd numbered experiments reveal that SCE frequencies are significant and clearly increase with increasing UV fluence in the fluence range 0-12 J/m^2. Comparison of the data of the control experiment (1 and 2) indicates that the PR fluence alone effects a relatively small increase in SCE frequency.

This increase is probably closely related to the photolysis of bromine in the BrdU labelled DNA (Hutchinson, 1973). Comparison of the data from experiments 3, 5, 7, 9, and 11 with the data of experiments 4, 6, 8, 10, and 12 reveals that the cells were capable of photoreactivating a relatively high level of UV-induced damage leading to SCEs; for example, approximately (9.10 - 2.80/ 9.10) .70 of the damage induced by 12.0 J/m^2 UV that leads to SCEs was photoreactivated. These data are consistent with those reported by Kato (1974), and imply that A87 cells can express a subset (of at least moderate size) of the pyrimidine dimers induced in their DNAas SCEs.

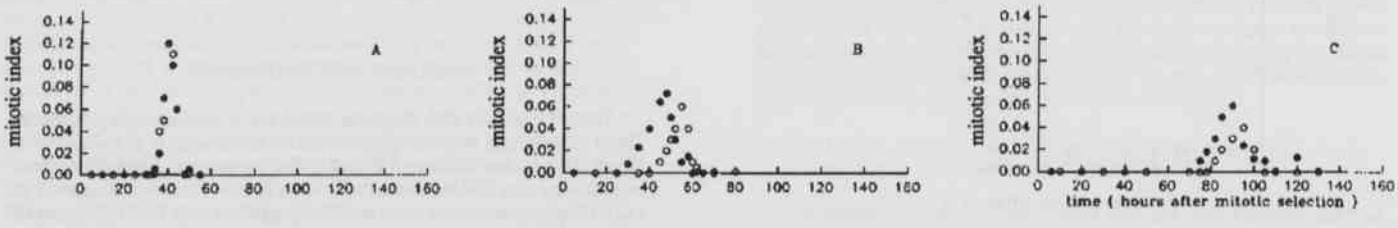

Figure 3. Mitotic index curves to determine the mitotic peaks at the first mitosis of the cells following (A) no UV fluence (filled circles) and 22,000 J/m^2 PR fluence only (open circles), (B) 1 J/m^2 UV fluence (filled circles) and 1 J/m^2 UV fluence + 22,000 J/m^2 UV fluence (open circles) and, (C) 12 J/m^2 UV fluence (filled circles) and 12 J/m^2 UV fluence + 22,000 J/m^2 PR fluence (open circles).

Previous detailed studies of the time course of PR of photoreactivable lethal lesions (PLLs) induced in early G1 phase *Xenopus* cells by UV (Griggs and Payne, 1981) indicated that these lesions could be efficiently photoreactivated shortly after the UV exposure, but as the damaged cells progressed through G1 phase they rapidly lost this ability. This observation, coupled with the data of Table 1, suggested our first attempt to enhance knowledge of the biological significance of SCEs. The attempt centered on experimentation designed to obtain an indication of the extent to which PSCEs in early G1 phase A87 cells constitute PLLs. It was reasoned that, if a substantial fraction of the PSCEs induced in early G1 phase by a given UV fluence are PLLs, then the kinetics for PR of these SCEs as a function of time following the UV exposure (i.e., time course of PR) would parallel the kinetics of the time course of PR of the PLLs induced by the PR fluences. Thus, time course of PR experiments of this nature were performed and the resulting data are displayed in Table 2 and 3. These experiments were composed of four steps: (1) sets of synchronous cultures of (BrdU labelled) G1 phase cells, one set for each experiment, were exposed to a UV fluence of 5.0 J/m^2 one hour after mitotic selection. (2) All but one (control) of these sets of UV irradiated cultures were then exposed to 22,000 J/m^2 PR light after the termination of UV at varying time intervals (a different interval for each set) following the exposure. (3) Both the UV + PR irradiated cultures were then allowed to progress to mitosis where samples of cells were collected from the peaks of mitotic activity by colcemid treatments for SCE analysis. Detailed parallel mitotic index curves, such as those of Figure 2, were established to describe the mitotic peaks. (4) The cells were scored and the number of SCEs per cell (Table 2) was determined (± standard error). A control (experiment 1) was established to determine the number of SCEs per cell before exposure to PR light. In experiments 2, 3, 4, and 5, the cells were given a PR fluence of 22,000 J/m^2 from 1 to 4 hours after UV, respectively. The number of SCEs decreased significantly. As the PR time was increased to 5 hours after UV (experiment 6), the photoreactivating capability of the cells appears to be blocked causing the number of SCEs to increase. This phenomenon was apparent through experiment 8, where the PR time was 7 hours after UV. In experiment 9, the number of SCEs began to decrease again (PR time being 8 hours) and this continued until the PR ability of the cells was no longer effective (early S phase). These data demonstrate that the pyrimicine dimers leading to SCEs are photoreactivable in early and late G1 phase, but are not photoreactivable in the middle of G1 phase. The mechanisms associated with this are not known at this time, but this could explain the difference in results that Kato (1974), Wolff (1978), and others obtained.

Table 2. Time course of PR of SCEs induced by irradiation of early G1 phase A87 cells with a UV fluence of 5.0 J/m^2.

Experiment Number*	PR fluence (J/m^2)	PR time (hrs after UV)	Number cells scored	Number SCEs per cell (± standard error)
1	0	0	250	6.25 (±1.21)
2	22,000	1	250	1.90 (±0.15)
3	22,000	2	250	1.83 (±0.16)
4	22,000	3	250	1.95 (±0.10)
5	22,000	4	250	1.75 (±0.09)
6	22,000	5	250	3.10 (±0.46)
7	22,000	6	250	4.90 (±1.02)
8	22,000	7	250	3.65 (±0.64)
9	22,000	8	250	2.35 (±0.21)
10	22,000	9	250	1.90 (±0.11)
11	22,000	10	250	1.88 (±0.10)
12	22,000	11	250	1.95 (±0.10)
13	22,000	12	250	3.85 (±0.44)
14	22,000	13	250	5.30 (±0.93)
15	22,000	14	200	6.15 (±1.16)
16	22,000	16	200	5.95 (±1.01)
17	22,000	20	200	6.40 (±1.22)
18	22,000	30	200	6.20 (±1.30)

* Synchronous cultures, unifiliarly labelled with BrdU, were exposed to UV one hour after mitotic selections. The mitotic indices of these cultures at the beginning of the experiments were greater than 0.97.

Table 3. Time course of PR of lethal lesions induced by irradiation of early G1 phase A87 cells with a UV fluence of 5.0 J/m^2.

Experiment Number*	PR fluence (J/m^2)	PR time (hrs after UV)	Number cells plated	Normalized surviving fraction (± standard error)
1	0	0	3,000	0.180 (±0.002)
2	22,000	0.5	3,000	0.940 (±0.014)
3	22,000	1.0	3,000	0.900 (±0.015)
4	22,000	1.5	3,000	0.880 (±0.003)
5	22,000	2.0	3,000	0.712 (±0.002)
6	22,000	2.5	3,000	0.650 (±0.001)
7	22,000	3.0	3,000	0.570 (±0.001)
8	22,000	3.5	3,000	0.475 (±0.002)
9	22,000	4.0	3,000	0.360 (±0.001)
10	22,000	4.5	3,000	0.280 (±0.001)
11	22,000	5.0	3,000	0.210 (±0.002)
12	22,000	5.5	3,000	0.179 (±0.001)
13	22,000	6.0	3,000	0.182 (±0.001)
14	22,000	7.0	3,000	0.175 (±0.001)
15	22,000	8.0	3,000	0.191 (±0.001)
16	22,000	9.0	3,000	0.180 (±0.001)
17	22,000	10.0	3,000	0.182 (±0.002)
18	22,000	12.0	3,000	0.177 (±0.001)
19	22,000	15.0	3,000	0.178 (±0.001)
20	22,000	20.0	3,000	0.183 (±0.002)

* Early G1 phase cells (unifiliarly labelled with BrdU) were exposed to UV one hour after mitotic selection.

Data from the time course of PR of lethal lesions experiments are depicted in Table 3. These experiments consisted of essentially the same four steps as was used for the time course of PR of SCEs experiments (Table 2), except the normalized surviving fraction (instead of the number of SCEs) was determined. Experiment number 1 (control) shows the surviving fraction to be 0.180 without the application of PR. In experiment number 2, PR was introduced 0.5 hours after UV and the surviving fraction increased significantly (to 0.940). PR was continually administered at specific times after UV and the surviving fraction was determined as shown in Table 3. The surviving fraction continued to decrease up to PR time 5.5 hours after UV. At this point, the pyrimidine dimers associated with lethal killing appear to be no longer photoreactivable. As the PR

time after UV was increased, the ability of the cells to photoreactivate continually dropped. This coupled with the data from Table 2 shows that the kinetics associated with PLLs are different from the kinetics associated with PSCEs, demonstrating that PSCEs constitute few, if any, lethal lesions. Plotting the fraction of maximum PR of SCEs against the fraction of maximum PR of lethal lesions (Figure 4) clearly indicates that the set of PPDs expressed as PSCEs are not identical with the set expressed as PLLs and implies that (1) the mechanisms associated with SCE induction are different from those associated with lethal lesions, and (2) further research should be attempted in this area to explain why the photoreactability of SCEs is blocked in the middle of G1.

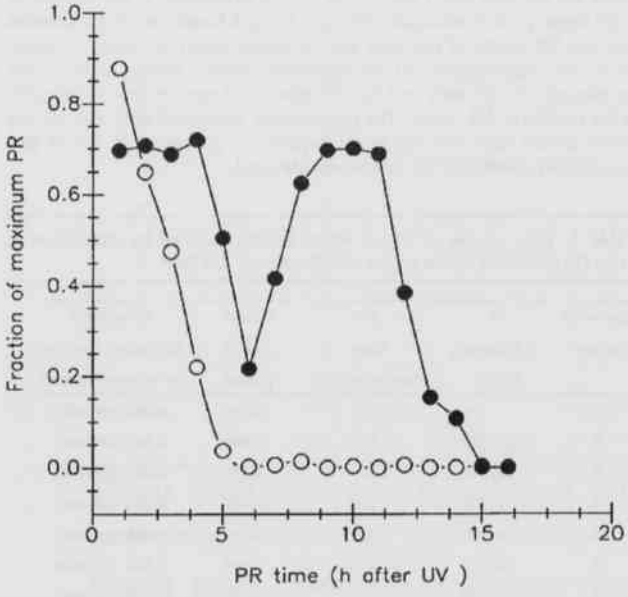

Figure 4. Time course curves showing how the fraction of maximum PR of UV-induced SCEs (filled circles) differs from the fraction of maximum PR of UV-induced lethal lesions (open circles) as the cells progress through G1 phase into S phase. The curves clearly indicate that the sets of PPds associated with each are not identical.

In conclusion, the experimentation described here indicates that a substantial fraction of the SCEs induced in early G1 phase A87 cells by UV are PSCEs and, thus, result from intracellular expression of pyrimidine dimers induced by the UV. Relatively few, if any, of these PSCEs constitute PLLs. This experimentation also suggests that, additional high resolution cytological studies of this nature may yield further interesting correlations between radiation repair mechanisms, changes in chromatin structure, and organization as cells progress through the cell cycle.

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