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Ultraviolet Light Reactivation of Gamma-Ray Induces Chromosome Aberrations in G1 Phase Xenopus Cells

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GENERAL NOTES

ULTRAVIOLET LIGHT REACTIVATION OF GAMMA-RAY INDUCED CHROMOSOME ABERRATIONS IN G1 PHASE *XENOPUS* CELLS*

Mitigation of ionizing radiation-induced effects by appropriate administration of low UV doses, termed ultraviolet light reactivation (UVR), has been observed in a number of prokaryotic (Weigle, 1953; Rupert and Harm, 1966) and lower eukaryotic (Elkind and Sutton, 1959; Calkins and Todd, 1968; Calkins and Griggs, 1969) cells. Cross and Griggs (1978) detected a significant level of UVR of gamma ray-induced lethal damage in a number of established vertebrate cell lines, including the A8W243 *Xenopus* line. We report here an attempt to determine whether UVR extends to chromosomal aberration induction by gamma ray in G1 phase cells of a line (A8W4) which was recently cloned from the A8W243 line.

Monolayers of A8W4 cells were routinely maintained at room temperature in plastic tissue culture flasks (Falcon) in HEPES buffered F10 medium (Gibco) supplemented with 10 percent fetal calf serum (Kansas City Biological). Synchronous cultures of early G1 phase cells were obtained by a miotic harvest method similar to the one described by Griggs and Orr (1979). Techniques employed for mitotic index determination, collection of mitotic cells by colcemid, and preparation of chromosome spreads for aberrational analysis were essentially the same as described by Griggs and Bender (1972), and Wolff (1962). Gamma ray was administered at a dose rate of 50 rads/minute by a Mark IV Cesium 137 irradiator as described by Cross and Griggs (1978). UV light (254nm) was administered at a dose rate of 5 ergs/mm²/sec. in the same manner as described by Griggs and Orr (1979). All experimentation was carried out at 25 °C under red light.

In the UVR experiment the desired doses of UV and gamma ray were administered to synchrounous cultures of G1 cells and, during postirradiation incubation, many of the exposed cells progressed through S and G2 to mitosis, where samples were collected with colcemid for chromosomal aberration analysis. Nonirradiated synchronous cultures of cells normally progress through interphase to mitosis in approximately 22 hours and retain a high degree of synchrony during their progression; however, administration of variable doses of UV and gamma ray to these cultures induces complex delays in the progression of the cells through S phase, and significantly lowers synchrony. Thus, detailed mitotic index experiments (see Figure) were carried out to determine the post irradiation peaks of mitotic activity and the corresponding time ranges for collection of appropriate samples of mitotic cells for aberration analysis.

As indicated by the chromatid-type aberration data of Table 1, administration of 200 rads gamma ray plus low UV doses (in the range 0-80 ergs/mm²) fails to induce significant frequencies of chromatid aberrations. This datum is consistent with studies indicating that ionizing radiation induces few, if any, chromatid aberration in G1 phase cells (Elkind and Whitmore, 1967), and the study by Griggs and Orr (1979) indicating that UV doses in the range 0-90 ergs/mm² fail to induce significant frequencies of chromatid aberrations in G1 phase A83 *Xenopus* cells. Comparison of the chromosome-type aberration data of Table 1 reveals that the various aberration frequencies vary as a complex function of UV dose. Rings and dicentrics appear to be subject to UVR, since the numbers of both types of aberrations decrease with increasing UV dose. In contract, the number of terminal deletions significantly increases with increasing dose. Results of the brief time course of UVR study (Table 2) indicate that the UV exposure must accompany, or be given shortly after, the gamma ray exposure to effect aberration production. Results of earlier studies by Wolff and Luippold (1955) indicate that most ionizing radiation induced chromosome breaks, including those involved in production of chromosome-type rings and dicentrics, rejoin or restitute shortly after formation. Their results, coupled with the data of Table 2, suggest that UV must be administered before the gamma ray induced breaks rejoin to effect significantly aberration production.

Interpretations of these data must be highly speculative, since radiation induced aberrant processes which lead to chromosome breakage, and intracellular processes which control rejoining of broken chromosome segments, are not understood (Elkind and Whitmore, 1967). Nevertheless, the following seems reasonable. Lesions induced in G1 chromosomes by low UV doses (in the range 0-80 ergs/mm²) produce few, if any, chromosome breaks, and have relatively little influence on the chromosome breakage induced by gamma ray. However, when these low UV doses are administered shortly before or after the gamma ray exposure, UV lesions induced in or near those chromosome sites where gamma ray induced breaks occur, significantly inhibit restitution of broken ends. Thus, the UV dependent increase in frequency of terminal deletions and decrease in frequencies of rings and dicentrics observed in the experiments of Table 1 may have resulted from UV inhibition of chromosome restitution processes.

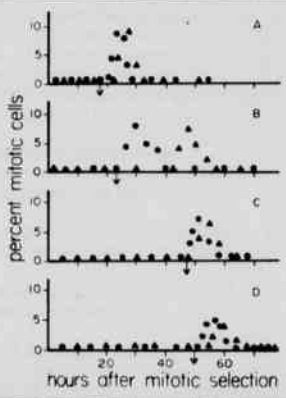

Figure. Percent mitotic cells as a function of time following irradiation of early (one hour old) G1 phase A8W4 cells with 200 rads gamma ray plus the following UV doses; 0 ergs/mm² A (circles), 10 ergs/mm² A (triangles), 20 ergs/mm² B (circles), 30 ergs/mm² B (triangles), 40 ergs/mm² C (circles), 50 ergs/mm² C (triangles), 60 ergs/mm² D (circles), 80 ergs/mm² D (triangles). Percent mitotic cells corresponding to time points to the left of downward pointing arrows were essentially zero.

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Table 1. UVR of gamma ray-induced chromosomal aberrations in G1 phase A8W4 cells as a function of UV dose. In each experiment synchronous cultures were exposed to the indicated doses of UV, one hour after mitotic selection, and 200 rads gamma ray was administered immediately after the UV exposure.

Experiment number	UV dose in ergs/mm^2	Collection time range (hours after mitotic selection)*	Number cells scored	Chromatid type aberrations		Chromosome type aberrations		
				Terminal deletions	Exchanges	Terminal deletions	Rings	Dicentrics
1	0	22-32	200	1	0	51	17	23
2	10	24-34	200	2	0	52	18	21
3	20	30-40	200	0	1	51	16	22
4	30	42-52	200	1	0	53	16	21
5	40	45-58	200	1	0	62	14	19
6	50	48-60	200	2	0	68	11	18
7	60	50-62	200	1	0	79	11	15
8	80	55-70	200	2	1	83	9	15

*Cells were collected for aberrational analysis by colcemid treatments that spanned the indicated time range.

Table 2. Time course of UVR of gamma ray-induced chromosome-type aberrations in A8W4 cells. In each experiment, a synchronous cultures of G1 phase cells was first exposed to 200 rads gamma ray (one hour after mitotic selection) and then exposed to 80 ergs/mm^2 UV, with the UV exposure beginning at the indicated time following termination of the gamma ray exposure.

Experiment number	UV dose (minutes after gamma ray exposure)	Collection time range (hours after mitotic selection)	Number cells scored	Chromosome type aberrations		
				Terminal deletions	Rings	Dicentrics
1	0	55-70	200	80	6	13
2	5	55-70	200	78	8	12
3	10	55-70	200	79	7	14
4	15	55-70	200	76	6	12
5	25	55-70	200	62	12	15
6	45	55-70	200	49	15	23
7	90	55-70	200	50	17	24

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THE WATER CRISIS — AN APPROACH FOR TEACHERS OF GRADES 7-12

The development of a water-ecology workshop has resulted from the confluence of three observations. First, several recent events attest a growing concern for the quantity and quality of Arkansas' water. In 1981 Governor Frank White appointed a committee to develop a comprehen-