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Time Course of PR of UV-Induced Chromosomal Aberrations and Lethal Damage in S and G2 *Xenopus* Cells

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ABSTRACT

S and G2 phase cells were exposed to 150 ergs mm^{-2} UV and their ability to photoreactivate the induced cell killing (loss of colony forming ability) and chromosomal aberrations was determined as a function of time following the UV exposure. In S phase cells, the lesions leading to cell death and those leading to aberrations were both converted to a non-photoreactivable state shortly after the UV exposure. A significant fraction of the lesions induced in G2 cells, that led to cell death, were converted to a non-photoreactivable state before the progeny of the exposed cells reached the next succeeding S phase. Few, if any, lesions were induced in G2 cells that were expressed as aberrations at the first mitosis following exposure. Some of the lesions induced in G2 cells led to aberrations that were observable in the progeny that progressed to the second mitosis following exposure. These lesions were converted to a non-photoreactivable state as the progeny of the exposed G2 cells progressed through the first S phase following exposure.

INTRODUCTION

Due primarily to its unusually efficient photoreactivation (PR) mechanism, the A8W243 *Xenopus* tissue culture cell line has proven to be a superior system for the study of many UV-induced effects in vertebrate cells (Griggs and Bender, 1972, 1973; Orr and Griggs, 1976). Recently Payne and Griggs (1977) reported a study with G_1 phase *Xenopus* cells in which this PR mechanism was used to examine the extent of overlap of UV-induced lesions that lead to chromosomal aberrations (aberrational lesions) and UV-induced lesions that lead to cell death (lethal lesions). Synchronous cultures of G_1 cells were irradiated with 120 ergs mm^{-2} UV. As the cells progressed through the cycle to the first succeeding mitosis (M1), the extent of PR of induced lethal and aberrational damage was determined as a function of time. A significant fraction of the lethal lesions was converted to a non-photoreactivable state while the cells were in G_1 phase, but most of the aberrational lesions were converted to a non-photoreactivable state as the cells entered S phase, indicating that many of the lethal lesions were not identical to aberrational lesions and that different intracellular mechanisms were responsible for their expression. We report here two sets of experiments which constitute an extension of these time course of PR studies with *Xenopus* cells; the first set was performed to examine the overlap of UV-induced lethal and aberrational lesions in S phase cells, and the second set was performed to examine the overlap of UV-induced lethal and aberrational lesions in G2 phase cells.

MATERIALS AND METHODS

The A8W243 *Xenopus* line used by Payne and Griggs (1977) was also utilized in this study. Routine procedures such as incubations, irradiations, DNA labelling with tritiated thymidine (^{3}HTdR), mitotic index determination, survival determinations, colcemid treatment, preparation of metaphase spreads, and chromosome scoring were the same as described earlier (Griggs and Bender, 1972, 1973; Wolff, 1961).

Experiments performed to describe the degree of overlap of lethal and aberrational damage induced in S cells by UV were carried out in essentially the same manner as the time course of PR experiments with G_1 cells reported by Payne and Griggs, 1977.

The procedures used in experiments to examine overlap of lethal and aberrational lesions induced in G2 cells by UV differed somewhat from those employed in experiments with S cells. Since no method was available for obtaining synchronous cultures of G2 cells, the starting point of these experiments was UV irradiation of vigorously growing log phase cultures. As the progeny of the G2 cells (in the exposed log phase cultures) progressed through M1 and the following cell cycle, attempts were made to describe the degree of overlap of lesions by time course of PR techniques. Synchronous cultures of G_1 cells, which were progeny of the exposed G2 cells that progressed through M1, were required in a number of experiments. These cultures were obtained by the following technique. Half the members of a set of vigorously growing log phase monolayers were labelled with ^{3}HTdR and, immediately afterwards, the entire set was exposed to 150 ergs mm^{-2} UV. Periodic agitation of these monolayers with a mechanical agitator yielded suspensions of cells with mitotic indices ranging from 50-70 percent. Autoradiographs prepared from suspensions obtained from labelled monolayers indicated that 99 percent of the mitotic cells were devoid of label, and thus derived from exposed G2 cells. The suspensions that contained no labelled cells were seeded into a large glass petri plate which contained BSS and fetal calf serum (1cc serum / 100cc BSS). At room temperature the cells quickly settled to the bottom of the plate but remained detached, and each cell which completed mitosis formed two small G_1 cells that remained in close proximity (double). These doubles could be identified and separated from other components of the suspension with the aid of a stereomicroscope and micropipette. New suspensions of early G_1 cells which exhibited a high degree of synchrony were thus obtained.

RESULTS AND DISCUSSION

Results of the time course of PR of lethal damage are shown in tional damage in early S phase cells are shown in Tables 1 and 2, respectively. The data of Table 1 indicate that the intracellular mechanism, which converts lethal lesions to a non-photoreactivable state, becomes functional shortly after the UV exposure, and the cells' ability to PR such lesions becomes negligible by the five hour point. The data of Table 2 indicate that the mechanism which converts aberrational lesions to a non-photoreactivable state also becomes functional shortly after the UV exposure; however, the cells still retain ability to PR a significant level of aberrational damage at the 12 hour point. These kinetics are quite similar to those reported by Payne and Griggs (1977) in their study with G_1 cells. They indicate that a significant fraction of the lethal and aberrational lesions induced in S cells is not identical, and they imply that different mechanisms are involved in their expression.

Results of the time course of PR of lethal damage are show in Table 3. Experiments 1-5 were performed to determine whether lethal damage was converted to a non-photoreactivable state during the first 3 hours following UV irradiation. No method was available for obtaining suitable samples of G2 and mitotic cells for the survival determinations in these experiments. However, the labelling and mitotic index data of Figure 1 indicate that virtually all of the G2 cells in the UV exposed log phase cultures progress to M1 without significant delay. Furthermore, routine mitotic index determinations of cell

Table 1. Time course of PR of lethal damage induced in early S phase cells by UV. UV was administered at a dose rate of 5 ergs mm^{-2} sec^{-1} and PR light was administered at a dose rate of 100 ergs mm^{-2} sec^{-1}

Experiment number	UV dose minutes	PR dose minutes	PR time (hrs after UV)	Number cells isolated	Number colonies assayed	Surviving fraction
1	0	0		2000	1602	0.80
2	0	45	0.25	2000	1584	0.79
3	30	0		2000	21	0.01
4	30	45	0.25	2000	1410	0.70
5	30	45	1.00	2000	980	0.49
6	30	45	2.00	2000	310	0.15
7	30	45	4.00	2000	98	0.05
8	30	45	5.00	2000	23	0.01
9	30	45	10.00	2000	28	0.01
10	30	45	15.00	2000	16	0.01
11	30	45	20.00	2000	19	0.01
12	30	45	24.00	2000	22	0.01

Table 2. Time course of PR of aberrational damage induced in early S phase cells by 150 ergs mm^{-2} UV. PR light was administered at a dose rate of 100 ergs mm^{-2} sec^{-1}.

Experiment number	PR time (hrs after UV)	PR dose (minutes)	Cell collection time range (hrs after UV)*	Number cells scored	Chromosomal aberrations: deletions	exchanges
1		0	80 - 95	500	310	271
2	0.25	45	18 - 33	500	20	8
3	0.50	45	20 - 35	500	26	10
4	1.00	45	40 - 55	500	31	12
5	2.00	45	45 - 60	500	38	14
6	4.00	45	55 - 70	500	61	29
7	8.00	45	60 - 75	500	112	71
8	12.00	45	70 - 85	500	241	214
9	15.00	45	70 - 90	500	307	272

*Metaphase cells were collected by colcemid treatments that spanned the indicated time ranges.

suspensions obtained by agitation of UV exposed log phase cultures, coupled with stereomicroscopic determinations of the fractions of cells in these suspensions that form 'doubles,' indicated that more than 98 percent of the mitotic cells completed M1. Thus, since practically all the UV exposed G2 cells progressed through M1, suitable sets of cells for the survival determinations in experiments 1-5 could be obtained from sets of early G_1 cells which were progeny of the treated G2 cells that progressed through M1. Progeny of the treated G2 cells that completed M1 were also the source of sets of cells isolated for survival determinations in experiments 6-12. The data of experiments 1-5 and Figure 1 indicate that little, if any, of the lethal damage can be photoreactivated as cells progress through G2 phase and early M1. The data of experiments 6-12 closely parallel results of the time course of PR of lethal damage in G_1 cells (Payne and Griggs, 1977), indicating that much of the lethal damage is converted to a non-photoreactivable state as progeny of the UV exposed G2 cells progress through the first G_1 phase following the exposure.

Results of the time course of PR of aberrational damage induced in G2 cells by UV are shown in Table 4. The data of experiments 1 and 2 indicate that the amount of aberrational damage expressed when the UV exposed cells reach M1 is negligible, as compared with the amount expressed when the progeny of the exposed cells reach M2. The data of experiments 3-11 closely parallel results of the similar study with G_1 phase cells (Payne and Griggs, 1977). Data of experiments 3-6 strongly suggest that aberrational damage could be photo-

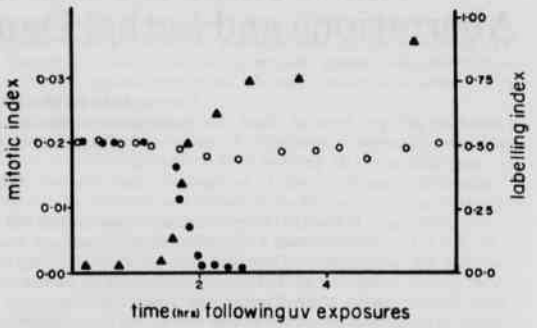

Figure 1. Mitotic indices (circles) and labelling index, percent labelled mitotic cells, (triangles) as a function of time following UV exposures to log phase cultures. Open circles represent cultures exposed to 0 ergs mm^{-2} UV (control). Filled circles represent cultures exposed to 150 ergs mm^{-2} UV. Triangles represent cultures pulse labelled with ^{3}HTdR immediately before being exposed to 150 ergs mm^{-2} UV.

reactivated in the progeny of the UV exposed G2 cells as these progeny progressed through the first G_1 phase following M1, while the data of experiments 7-11 suggest that aberrational damage is converted to a non-photoreactivable state as the progeny progress through the first S phase following M1.

A suitable explanation for the observation that G2 cells have little, if any, PR ability cannot be deduced from the data presented here. Further elucidation of the manner in which PR related to chromosome structure appears to be required. One might conjecture at this point, however, that the chromosome supercoiling and condensation processes operating in G2 cells (Prescott, 1970) might significantly decrease PR ability by somehow preventing normal complexing of PR enzyme with UV-induced dimers in DNA.

Table 3. Time course of PR of lethal damage induced in G2 cells by UV. A UV dose rate of 5 ergs mm^{-2} sec^{-1} and a PR dose rate of 100 ergs mm^{-2} sec^{-1} were used.

Experiment number*	UV dose minutes	PR dose minutes	PR time (hrs after UV)	Number cells isolated	Number colonies assayed	Surviving fraction
1	30	0		3000	274	0.09
2	30	45	0.25	3000	281	0.09
3	30	45	1.00	3000	273	0.09
4	30	45	1.50	3000	278	0.09
5	30	45	2.50	3000	280	0.09
6	30	45	3.50	3000	1240	0.40
7	30	45	4.00	3000	1100	0.36
8	30	45	5.00	3000	1012	0.33
9	30	45	7.00	3000	714	0.23
10	30	45	9.00	3000	422	0.14
11	30	45	11.00	3000	276	0.09
12	30	45	13.00	3000	280	0.09

*In experiments 2-4, UV irradiated G2 cells were photoreactivated before they reached M1, and the sets of cells isolated were subsets of the populations of early G_1 cells, which were produced as the UV exposed cells completed M1. In experiments 5-10, progeny (of UV exposed G2 cells that progressed through M1) were photoreactivated at the times indicated, and cells were isolated immediately after PR.

Table 4. Time course of PR of aberrational damage induced in G2 cells by 150 ergs mm^{-2} UV. PR light was administered at a dose rate of 100 ergs mm^{-2} sec^{-1}.

Experiment number*	PR time (hrs after UV)	PR dose (minutes)	Cell collection time range (hrs after UV)**	Number cells scored	Chromosomal aberrations	
					Deletions	Exchanges
1		0	0 - 3	500	2	1
2		0	65 - 80	500	149	92
3	4.50	45	28 - 40	500	14	6
4	6.00	45	32 - 44	500	15	4
5	7.00	45	34 - 48	500	12	5
6	8.00	45	40 - 55	500	13	8
7	10.00	45	45 - 60	500	87	43
8	12.00	45	58 - 73	500	138	96
9	15.00	45	60 - 75	500	146	83
10	20.00	45	65 - 80	500	151	88
11	40.00	45	65 - 80	500	142	99

*M1 metaphase cells were analyzed in experiments 1, while M2 metaphase cells were analyzed in experiments 2-10.
**Metaphase cells were collected by colcemid treatments that spanned the indicated time ranges.

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