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Time Course of Photoreactivation of UV Induced Damage in G1 Phase Xenopus Cells That Leads to Chromosome Breaks Observable by Premature Chromosome Condensation

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TIME COURSE OF PHOTOREACTIVATION OF UV INDUCED DAMAGE IN G1 PHASE XENOPUS CELLS THAT LEADS TO CHROMOSOME BREAKS OBSERVABLE BY PREMATURE CHROMOSOME CONDENSATION

In a study of the time course of photoreactivation (PR) of UV-induced primary lesions in the DNA of G1 phase *Xenopus* cells, Griggs and Payne (1981) observed that most of the lesions that led to chromosomal aberrations could be efficiently photoreactivated while the cells progressed through G1 phase. However, these lesions appeared to somehow be converted to a nonphotoreactivable state as the cells entered S phase. Since, in chromosomal aberration production, the chromosome strands are apparently first broken and then the resulting fragments are improperly rejoined or left unattached, and since PR of aberrations requires that administration of photoreactivating light precede the chromosome breakage, these observations by Griggs and Payne (1981) were interpreted as follows: Most of the photoreactivable UV-induced primary lesions in the DNA of G1 phase *Xenopus* cells that lead to chromosome breaks lie essentially dormant as the cells progress through G1 phase. These lesions are expressed as chromosome breaks in the cells as they enter S phase, perhaps by some mechanism associated with unwinding of the double helix for DNA synthesis. Chromosomal aberrations observable in the cells when they reach the first succeeding mitosis result from improper joining of broken chromosomal fragments. We briefly describe here the first of a set of studies designed to examine the validity of this interpretation.

Among the implications of the interpretation are the following: (1) If UV irradiated G1 phase *Xenopus* cells could be following: (1) If UV irradiated G1 phase *Xenopus* cells could be examined periodically for chromosome breaks as they progressed through interphase, no significant frequency of chromosome breaks would be observed until the cell entered S phase. (2) The frequency of these UV-induced breaks could be decreased by PR only if the PR light was administered to the cells while in G1 phase. We recently developed a premature chromosome condensation (PCC) technique for observing interphase *Xenopus* chromosomes making it possible to test these implications experimentally.

The cell line used was the A86 *Xenopus* line described by Kulp and Griggs (1989). This line has an exceptionally stable karyotype of 36 chromosomes (Fig. 1). Techniques used for obtaining synchronous cultures of G1 phase cells, incubations, UV and PR irradiations, tritiated thymidine flash labelling, preparation of autoradiographs, and mitotic index determinations were the same as described in detail by Griggs and Bender (1972, 1973), Griggs and Orr (1979), and Griggs and Payne (1981). The PCC technique developed was similar to those described for scoring breaks in interphase mammalian chromosomes by Waldren and Johnson (1974), and Pantelias and Maillie (1985). A typical G1 phase PCC cell (cell with prematurely condensed chromosomes) is shown in Fig. 2.

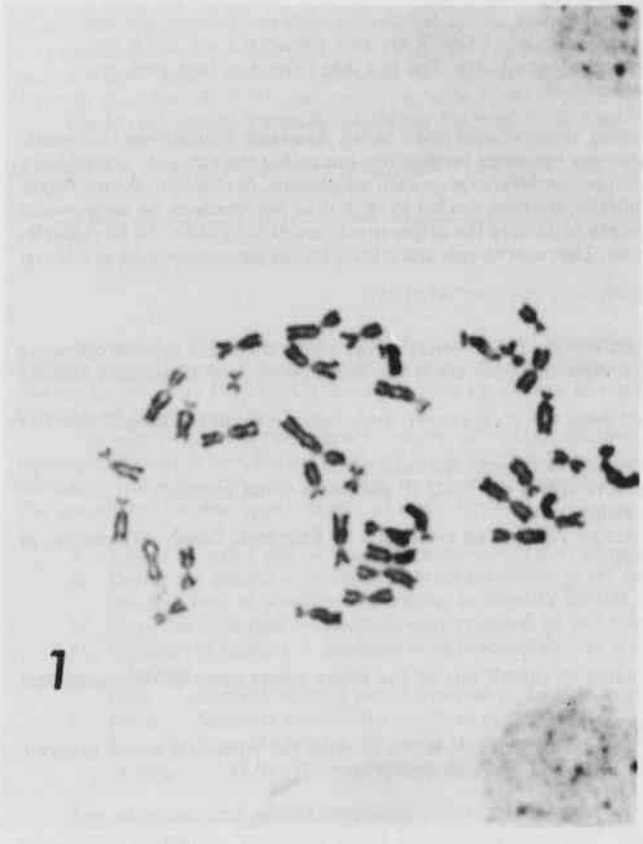

Figure 1. A typical metaphase spread for normal A86 *Xenopus* cells.

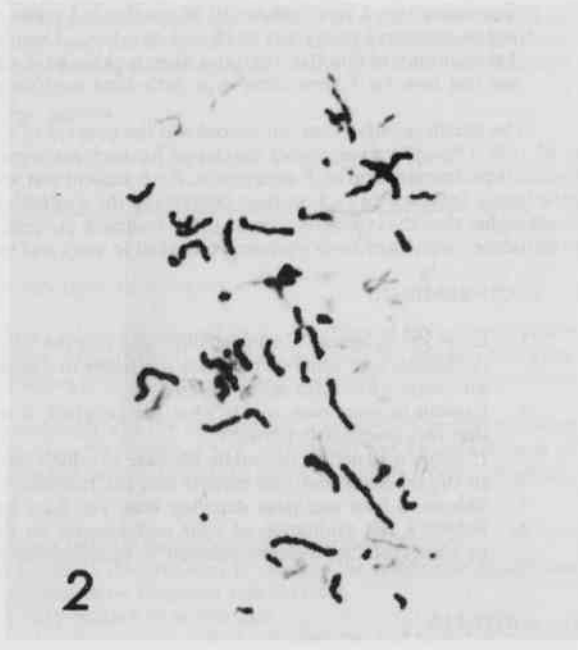

Figure 2. A PCC A86 *Xenopus* G1 phase chromosome spread. The differential chromosome staining technique, developed by Perry and Wolff (1974), was used to stain the G1 phase monopartite (single stranded) chromosomes more deeply than the bipartite metaphase chromosomes.

Results of the experiments are shown in Table 1. The starting point for each experiment was UV-irradiation of synchronous cultures of G1 phase cells, 4 hours after mitotic selection. As these irradiated cells progressed through the remainder of G1 phaSe and part of S phase, they were photoreactivated, flash labelled with ^{3}HTdR and subjected to PCC as indicated in Table 1. Autoradiographs of the cells were then prepared on microscope slides and PCC cells were selected at random from these slides and scored for chromosome breaks.

Three particularly relevant observations can be made by focusing on the data of certain subsets of the experiments: (1) Comparison of the data of experiments 1-4 with that of experiments 5-10 reveals that the radiation fluences administered in the latter experiments did not significantly

General Notes

Table 1. Time course of appearance of chromosomal breaks in *Xenopus* cells that were exposed to UV in G1 phase, and time course of PR of these breaks.

Experiment number	UV fluence (J/m^2)*	PR fluence (J/m^2)	PR time (hrs after UV)	3HTdR labelling time (hrs after UV)	Number of PCC cells scored**	Number of breaks scored per cell	Percent of PCC cells labelled
1	0	0		2.50	150	0.012	0
2	0	0		3.00	150	0.011	4
3	0	0		3.50	150	0.010	5
4	0	0		4.00	150	0.012	96
5	18.0	0		2.50	150	0.010	6
6	18.0	2×10^4	0.5	2.50	150	0.010	7
7	18.0	0		3.00	150	0.015	5
8	18.0	2×10^4	0.5	3.00	150	0.012	6
9	18.0	0		4.00	150	0.115	95
10	18.0	2×10^4	0.5	4.00	150	0.030	96
11	18.0	0		5.00	150	0.125	94
12	18.0	2×10^4	0.5	5.00	150	0.025	95
13	18.0	0		7.00	150	0.115	96
14	18.0	2×10^4	7.5	7.00	150	0.120	95
15	18.0	0		10.00	150	0.120	94
16	18.0	2×10^4	10.5	10.00	150	0.110	94

* UV was administered 4 hours after mitotic selection. ** PCC was induced shortly after labelling and PR.

influence the progress of the cells through G1 phase into S phase; furthermore, the cells entered S phase about 3-3.5 hours after the UV exposure. (2) Consideration of the data of the control experiments (1-4) in conjunction with the data of the experiments in which the cells received UV but no PR light (5, 7, 9, 11, 13, and 15), reveals that the number of chromosome breaks observed in the latter experiments exceeded that observed in the control experiments only after about 4 hours past the UV exposure (experiment 9); which (by observation 1) was after the cells had entered S phase. Observation 1 combined with observation 2 appears to adequately confirm implication 1. (3) Comparison of the data of experiments 5, 7, 9, 11, 13, and 15 with the data of experiments 6, 8, 10, 12, 14, and 16, respectively, indicates that UV-induced chromosome breaks were photoreactivated only in experiments 10 and 12 in which PR light was administered 0.5 hours after the UV exposure; which by observation 1 was during the G1 phase. Thus, observation 1 combined with observation 3 appears to confirm implication 2.

In conclusion, the results of the experimentation described here tend to support the interpretation by Griggs and Payne (1981) by confirming two of its implications. These results also suggest that additional appropriate experimentation of this nature may further strengthen the interpretation.

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Correction — In the article "Reproductive characteristics of south Florida *Sternotherus odoratus* and *Kinosternon baurii* (Testudines: Kinosternidae)" by Walter E. Meshaka that appeared in Volume 42 (1988) of the Proceedings of the Arkansas Academy of Science, pages 111-112, the following correction should be noted. In the third paragraph, the first sentence should read "Average carapace lengths for eighteen sexually mature females (77 + 5.69; range = 62-86) and ten sexually mature males (68 + 8.79; range = 52-80) support Tinkle's (1961) findings of sexual dimorpism in southern populations."