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Joel Staggers
John Brown University

H. Gaston Griggs
John Brown University

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GENERAL NOTES

PHOTOREACTIVATION OF CHROMATID DELETIONS INDUCED BY UV-IRRADIATION OF G1 PHASE HAMSTER X *XENOPUS* HYBRID CELLS

Some amphibian cells are replete with photoreactivating enzyme and are capable of photoreactivating a relatively high level of UV-induced division delay damage, lethal damage and damage leading to chromosomal aberrations (Biological photoreactivation) (Regan *et al.*, 1968; Griggs and Bender, 1972; Griggs and Orr, 1979; Griggs and Payne, 1981). Biological photoreactivation has not been clearly demonstrated in placental mammalian cells (Cleaver, 1974). However, relatively low levels of photoreactivating enzyme activity (removal of pyrimidine dimers from DNA) have been detected in some mammalian cells (Sutherland *et al.*, 1974; Sutherland *et al.*, 1976). Recently, Kulp *et al.* (1985) and Bohlender *et al.* (1987) attempted to elucidate the differences in amphibian and mammalian photoreactivation (PR) mechanisms by studying interactions of the mechanisms in the photoreversal of UV-induced damage leading to cell death in hamster (V79) X *Xenopus* (A8) hybrid cell lines. It was anticipated that the *Xenopus* genomes in the hybrids would produce PR enzyme in sufficient concentration and of such a nature as to efficiently photoreactivate UV-induced lethal damage (pyrimidine dimers) in both hamster and *Xenopus* DNA, and the level of PR observed for the hybrid lines would closely approach the high level observed for the *Xenopus* parental lines. To the contrary, the levels of PR exhibited by the hybrids did not closely approach that of the *Xenopus* lines. To assist in the interpretation of this unexpected observation, Bohlender *et al.* (1987) compared the levels of PR of chromatid deletions induced by UV in selected hamster and *Xenopus* chromosomes of the hybrid. The results suggested that the hybrid lacked the ability to efficiently photoreactivate deletions in hamster chromosomes; implying that the relatively low level of PR of lethal damage manifested by the hybrid cells might be a consequence of their inability to efficiently PR UV-induced primary damage (pyrimidine dimers) in hamster DNA. However, the key data supporting this suggestion were indirect and limited, being results of an experiment to compare the percentages of deletions induced by UV in a marker hamster chromosome and a marker *Xenopus* chromosome (of equal length) that could be photoreactivated. Since not all segments of vertebrate DNA of equal length appear to be equally accessible to the induction of UV damage or associated repair (e.g., Smith, 1987), the question of the extent to which the results found with this pair of heterologous chromosomes are representative of the entire hybrid genome appropriately arises.

It was assumed that an enlightening (if not convincing answer to this question might be obtained if, following a given UV or UV + PR treatment of a set of hybrid cells, the sum total of the chromatid deletions occurring in the hamster chromosomes of the set could be compared with the sum total of chromatid deletions occurring in the *Xenopus* chromosomes of the set. We describe here an attempt to effect such comparisons.

Performance of the comparisons required a dependable technique for distinguishing hamster chromosomes from *Xenopus* chromosomes in the same hybrid metaphase spread. The technique developed was a modification of conventional Giemsa banding techniques. Briefly hybrid metaphase spreads on microscope slides were subjected to the following three treatments in the order listed: (1) Incubate for 90 minutes at 60° C in 2 X SSC (17.5g NaCl + 8.8g sodium citrate per liter distilled water), then air dry). (2) Incubate for 15 minutes at 4° C (ice bath) in trypsin solution (1:300 crude power in 0.9% NaCl in distilled water). (3) Stain for 10 minutes in 2% Giemsa (Gibco). This technique adequately distinguishes the hamster chromosomes from the *Xenopus* chromosomes in hybrid cells, as illustrated in Figure 1.

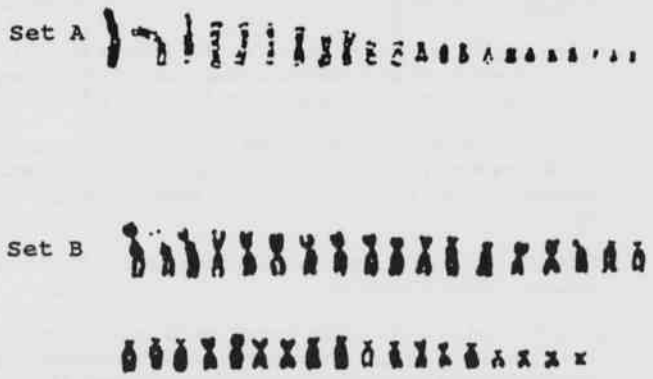

Figure 1. Differential Giemsa banding of the karyotype of a typical V79B3 (hamster) X A86 *(Xenopus)* hybrid cell. The banding technique was developed to distinguish V79B3 chromosomes (A, banded), from A86 chromosomes (B, not banded) in hybrid metaphase spreads.

Table 1. PR of chromatid deletions induced by UV in the chromosomes of G1 phase V79B3 X A86 hybrid cells.

Experiment number*	UV fluence (J/m^2)	PR fluence (J/m^2)	Number cells scored	Mean number of deletions/trial (± standard errors) observed in V79B3 chromosomes	Mean number of deletions/trial (± standard errors) observed in A86 chromosomes
1	0	0	1500	4	2
2	0	25,000	1500	3	3
3	5.0	0	1500	71.2 ± 9.1	119.2 ± 12.3
4	5.0	25,000	1500	67.2 ± 6.1	28.6 ± 5.1
5	10.0	0	1500	260.3 ± 11.1	420.4 ± 16.1
6	10.0	25,000	1500	240.4 ± 7.8	70.1 ± 10.2
7	15.0	0	1500	441.5 ± 17.3	701.2 ± 24.6
8	15.0	25,000	1500	381.2 ± 14.1	93.4 ± 7.1

*Each experiment consisted of three trials with 500 cells scored in each trial.

Conventional techniques for cell synchronization, irradiations and aberration analysis (Griggs and Orr, 1979; Griggs and Payne, 1981) were coupled with the banding technique to perform experiments for comparing frequencies of chromatid deletions occurring in the hamster and *Xenopus* chromosomes of sets of V79B3 X A86 cells, which were exposed to UV and/ UV + PR in G1 phase. Results of these experiments are shown in Table 1. The V79B3 X A86 line possessed a stable karyotype throughout the experimentation, with approximately 96% of the cells containing the entire complements of hamster and *Xenopus* chromosomes (22 and 36 respectively); however, as has been the case with previous hamster X *Xenopus* lines, the V79B3 X A86 line began to lose hamster chromosomes after about 100 cell passages (Kulp *et al.*, 1985). The PR scheme used in these experiments was essentially the same as the "optimum" PR scheme for the line studied by Bohlender *et al.* (1987) (i.e., Temperature-24° C; fluence rate-10w/m^2; total fluence-25,000 J/m^2). Comparison of the results of experiments 3, 5, and 7 indicates that a given fluence of UV induces a substantially higher number of deletions in the *Xenopus* chromosomes than in the hamster chromosomes of a set of hybrid cells; e.g. in experiment 3, hamster deletions/*Xenopus* deletions is approximately 71/119 = .60. However, when the sum of the lengths of the A86 chromosomes and the sum of the length of the V79B3 chromosomes, found in a set of hybrid metaphase spreads, were determined by ocular micrometer (data not shown), and compared; the ratio (sum of V79B3 lengths/sum of A86 lengths) was approximately .57. Thus, if it is assumed that segments of *Xenopus* and hamster G1 phase chromosomes of equal length contain equal lengths of vertebrate DNA, then *Xenopus* DNA and hamster DNA would appear to be about equally sensitive to chromatid deletion induction by UV. Comparison of the results of experiments 3, 5, and 7 with the results of experiments 4, 6, and 8 respectively, indicate that the V79B3 X A86 cells photoreactivated a much higher level of UV-induced damage leading to deletions in the A86 chromosomes than in the V79B3 chromosomes. For example, results of experiments 5 and 6 indicate that 1- 70/420 = .83 of the deletions in the A86 chromosomes were photoreactivated, while only 1- 240/260 = .08 of the deletion in the 79B3 chromosomes were photoreactivated.

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In conclusion, the data described here constitute an extension of the observation by Bohlender *et al.* (1987) on PR of UV-induced chromatid deletions in hamster X *Xenopus* hybrid cells, and support the notion that PR mechanisms in vertebrate cells do not remove pyrimidine dimers from all vertebrate DNA's with equal efficiency.

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JOEL STAGGERS and GASTON GRIGGS, Department of Biology, John Brown University, Siloam Springs, AR 72761.

A PRELIMINARY SURVEY OF THE COLLEMBOLA OF MAGAZINE MOUNTAIN, LOGAN CO., ARKANSAS

The order Collembola includes small, wingless insects with characteristic abdominal appendages and mouthparts that are enclosed within a gnathal pouch. The first, third and fourth segments of the six-segmented abdomen are modified to form the specialized jumping organs characteristic of the order. Collembola range in size from .25 mm to 1 mm. Development is direct in that newly eclosed individuals differ from adults only in size, body proportions and usually absence of pigment. Collembola molt throughout life, the number of instars ranging from two to 50. They may be found in any habitat from near polar conditions and extreme high altitudes to sea level (Christiansen and Bellinger, The Collembola of North America, North of the Rio Grande, Grinnell College, Grinnell, Iowa, 1981. p. 20). Classification is based almost entirely on external morphology and mouthpart structure.

Little information was available about the order in Arkansas. Previous work in Arkansas has shown 15 species to occur in the state (Christiansen and Bellinger, 1981, pp. 495-1110). There have been lists of collembolan fauna published from 10 other states (Christiansen and Bellinger, 1981). The Tennessee list (Copeland, T.P., A Preliminary LIst of the Collembola of East Tennessee, Journal of The Tennessee Academy of Science, 35[4] October, 1960, pp. 238-243) recorded 34 genera and 77 species. To further investigate the collembolan fauna in Arkansas, berlese samples were taken from seven study sites representing a diversity of habitats in the Magazine Mountain area from October 1985 through September 1988. The study sites consisted of: north-facing blufs with limestone outcroppings, sphagnum moss from a boggy area, a dry upland forest trail, rotting leaf litter along a small stream, moss samples, rotting logs, a drainage ravine with deep forest liter, and a hallow, rotting stump. Collembola were collected from the samples, cleared and slide mounted according to the methods outlined by Christiansen and Bellinger (1981) to facilitate examination under oil immersion (1000X). Approximately 1500 slides have been processed and classified from the sites. Findings include members from five families, 28 genera and 59 species. This represents 57 new state records. Voucher collections will be placed in the University of Arkansas Insect Collection. Determinations were made by the authors and by Drs. Kenneth Christiansen, Peter F. Bellinger and Richard J. Snider.

SPECIES LIST

Suborder: Arthropleona
Family: Hypogasturidae

Anurida (Micranurida) harti Christiansen and Bellinger
Hypogastura (Ceratophysella) armata (Nicolet)
Hypogastura (Ceratophysella) denticulata (Bagnall)
Hypogastura (Ceratophysella) glancei (Hammer)

Family: Entomobryidae

Entomobrya (Entomobrya) multifasciata (Tullberg)
Orchesella celsa Christiansen and Tucker
Orchesella villosa (Linnaeus)
Lepidocyrtus pallidus Reuter
Pseudosinella violenta (Folsom)