REPAIR OF UV-INDUCED PYRIMIDINE DIMERS IN *DROSOPHILA MELANOGASTER* CELLS IN VITRO*

JAMES E. TROSKO† AND KATHY WILDER

Department of Human Development, Michigan State University, East Lansing, Michigan 48823

Manuscript received August 24, 1972
Revised copy received October 25, 1972
Transmitted by DAVID SCHLESSINGER

ABSTRACT

Tissue culture cells of *Drosophila melanogaster* were given various doses of ultraviolet light. The results indicate that Drosophila cells do have a dark-repair excision mechanism which is not sensitive to caffeine. Pyrimidine dimers were destroyed by photoreactivating illumination in these cells and this destruction probably represents monomerization of the pyrimidine dimers.

THE detailed molecular basis for mutagenesis in either prokaryotes or eukaryotes has not been delineated. However, experimental progress has implicated the non-repair of DNA lesions or the type of enzymatic mechanism involved in DNA repair with the mutation process (WITKIN 1969; BRIDGES 1969).

Drosophila melanogaster continues to be one of the most convenient and well-studied eukaryotic organisms in mutagen testing. Therefore, with recent availability of Drosophila cells in tissue culture (SCHNEIDER 1971), it is now technically feasible to characterize some DNA repair mechanisms.

Several investigations have examined ultraviolet radiation effects on killing and mutagenesis (DEMEREC *et al.* 1942; GOLDMAN and SETLOW 1956; PRUDHOMMEAU 1972), as well as the effect of photoreactivation on UV-induced mutagenesis and phenocopy induction in Drosophila (ALTENBURG and ALTENBURG 1957; PERLITSCH and KELNER 1953; BROWNING and ALTENBURG 1962). Several unsuccessful attempts have been made to detect the photoreactivation enzyme in Drosophila (MUHAMMED and TROSKO 1967; COOK 1970). This report includes the demonstration of direct photoreactivation and the dark repair-excision of UV-induced pyrimidine dimers in Drosophila cells grown *in vitro*.

MATERIALS AND METHODS

Tissue Culture: Tissue culture cells of *Drosophila melanogaster* derived from embryos by DR. I. SCHNEIDER (line 2), obtained from DR. GEORGE MIKLOS (University of California, San Diego), were used for all the following experiments. The cells were grown at 25°C in Schneider's

* This investigation was supported by U.S. A.E.C. Contract AT-(11-1)-1704, by Public Health Service Research Grant No. CA 13048-01 from the National Cancer Institute and by N.I.H. Research Career Development Award 1 K04 CA 24085-01 to DR. TROSKO.

† On leave of absence at the McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706.

medium-revised (Grand Island Biological), supplemented with Difco Bacto-peptone (500 mg/100 ml) and with 15% calf serum. Cells were plated in 60 mm Falcon plastic petri dishes for experiments.

Irradiation Procedures: All the UV irradiations were performed with a germicidal lamp (G 25T8, delivering primarily 254 mμ light), mounted in a Microvoid transfer hood. The incident dose rate to the surface of the 1/2 ml Drosophila Ringer's solution containing the cells in a 60 mm petri plate was 25 ergs/mm²/sec (dose rate determined by a YS1 Model 65 radiometer, Yellow Springs Instruments, Yellow Springs, Ohio). After UV irradiation, the Ringer's solution was decanted and 3 ml of Schneider's media were placed on the cells to be tested for dark repair. The cells were placed in a dark incubator for twenty-four hours. Photoreactivaitng light was delivered by three General Electric black-light lamps (GE-F15T8-BLB; 320–450 mμ, predominantly 360 mμ) and one standard 15 watt daylight fluorescent bulb. Lamps were positioned to give a flux of 4800 ergs/mm²/minute through 1 cm of plate glass. Cells that were photoreactivated after UV irradiation were placed in 1.5 ml of the Schneider's medium for the radiation exposure.

Pyrimidine Dimer Assay: To analyze pyrimidine dimers in these Drosophila cells, the following procedures were used. Drosophila cells were inoculated into 60 mm Falcon plastic petri dishes (1×10^6 cells/plate). After one day's growth, fresh medium with tritiated thymidine (10 μc/ml; 53.4 Ci/mM, New England Nuclear) was added. After two days' incubation in the radioactive medium, non-radioactive medium was placed on the cells for 4 hours. Prior to irradiation, the medium was decanted from the plates, the edge of the monolayer of cells was scraped with a rubber "policeman" to remove cells that would be shadowed by the edge of the petri dish and the cells were carefully washed twice with Drosophila Ringer's solution. The cells were irradiated in 0.5 ml of the Ringer's solution. Immediately after the irradiation, the cells were either harvested or re-incubated in the dark in medium or photoreactivated. After the respective radiation or post-irradiation treatment, cells were fixed in cold 5% trichloroacetic acid and the insoluble residue was analyzed for pyrimidine dimers by two -dimensional chromatography after hydrolysis in formic acid (CARRIER and SETLOW 1971).

RESULTS AND DISCUSSION

Results of the experiments, designed to examine the ability of Drosophila cells, in vitro, to dark repair UV-induced pyrimidine dimers, are shown in Table 1. Pyrimidine dimers are produced linearly within the dose range given to these cells. These data are consistent with the interpretation that these cells were able to excise pyrimidine dimers from their DNA. Because of the technical difficulties of measuring the excised pyrimidine dimers in the tissue culture medium after the twenty-four-hour dark incubation, we did not check for the appearance of pyrimidine dimers in medium as they were disappearing from the TCA-insoluble fraction of the cells. At relatively low doses, approximately 40% of the pyrimidine dimers were removed, whereas at the high dose point, 90% of the pyrimidine dimers are still associated with the TCA-insoluble material. However, the data indicate that a constant number of pyrimidine dimers is excised in 24 hours. This observation has been observed before in bacterial and human cells (SETLOW 1964; CLEAVER and TROSKO 1970; SETLOW *et al.* 1969).

Caffeine, given as a posttreatment to UV-irradiated bacteria, synergistically increases the UV-induced killing and mutation frequencies (WITKIN 1958; LIEB 1951) by inhibiting the excision of pyrimidine dimers (LUMB, SIDEROPOULOS and SHANKEL 1968; SETLOW and CARRIER 1968; SIDEROPOULOS and SHANKEL 1968). The results in Table 2 indicate that the excision of UV-induced pyrimi-

TABLE 1

Dark repair (DR) of ultraviolet light-induced pyrimidine dimers ($\hat{XT}$) in* Drosophila melanogaster *cells grown in vitro*

Experiment	UV treatment	0 hours: Counts in $\hat{XT}$‡ / Counts in T	0 hours: $\frac{\hat{XT}}{T}$ (%)	24 hr-DR†: Counts in $\hat{XT}$ / Counts in T	24 hr-DR†: $\frac{\hat{XT}}{T}$ (%)
A.	None	0/474,000	0.00		
	15 sec§	680/931,000	0.07	110/415,000	0.03
	20 sec	660/612,000	0.11	350/602,000	0.06
	30 sec	1,450/1,015,000	0.14	350/552,000	0.06
	45 sec	.....	...	450/267,000	0.17
	60 sec	2,250/787,000	0.28	290/110,000	0.26
B.	15 sec	260/241,000	0.11	140/198,000	0.07
	20 sec	890/647,000	0.14	180/215,000	0.09
	30 sec	780/320,000	0.24	710/352,000	0.20
	60 sec	1,230/312,000	0.39	560/162,000	0.35

* $\hat{XT}$ refers to both $\hat{TT}$ and $\hat{UT}$ dimers, since chromatography procedures used here do not separate them from each other.

† DR refers to post radiation dark repair incubation.

‡ 10-minute counts were made of each sample. A background count of 30 cpm was subtracted from each sample.

§ Cells were irradiated at 254 mμ. The dose rate for experiment A was not determined accurately. The incident dose rate in experiment B was 25 ergs/mm²/sec.

dine dimers in Drosophila cells is not inhibited by caffeine posttreatment. The same result is noted in human cells (REGAN *et al.* 1968).

Figure 1 illustrates the results of photoreactivation treatment on UV-irradiated Drosophila cells. The fact that these cells did not excise a significant fraction of pyrimidine dimers at the high dose range in twenty-four hours indicates that the pyrimidine dimers were destroyed rather than excised. Photoreactivating enzyme activity has been demonstrated in the arthropod, *Anagasta kühniella* (Flower

TABLE 2

Lack of an effect of caffeine on the excision of UV-induced pyrimidine dimers Drosophila melanogaster *cells in vitro**

Experiment	Treatment	Counts in $\hat{XT}$ / Counts in T	$\frac{\hat{XT}}{T}$ (%)
C.	UV alone	560/427,000	0.13
	UV + 24 dark incubation	370/513,000	0.07
	UV + 1×10^{-3} M Caffeine-dark incubation	320/421,000	0.07
D.	UV alone	1,080/937,000	0.12
	UV + 24 dark incubation	340/400,000	0.08
	UV + 1×10^{-3} M Caffeine-dark incubation	180/267,000	0.07

* Cells were irradiated with 254 mμ UV light. Total incident dose was 500 ergs/mm² at a dose rate of 25 ergs/mm²/sec. A background count of 28 cpm was subtracted from each sample; 10-minute counts were made on each sample.

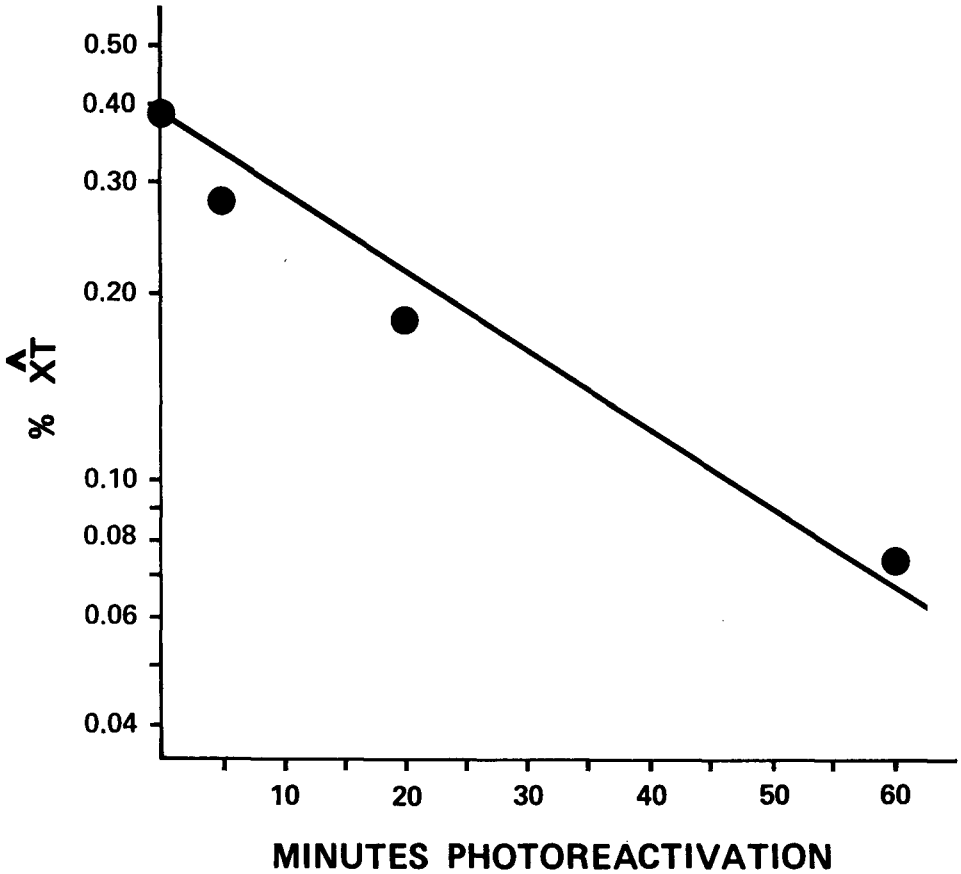

FIGURE 1.—Light-dependent dimer reversal in vitro in *Drosophila melanogaster* cells which received 1500 ergs/mm^2 of 254 mμ UV. Ordinate: percent of total thymine as thymine-or uracil-thymine dimer (XT). Abscissa: time in photoreactivating light at 25°C.

moth) (COOK and MCGRATH 1967). Together with the latter observation and with observations of biological photoreactivation in *Drosophila*, we infer that the destruction of pyrimidine dimers is due to the monomerization of the dimers.

These results indicate that *Drosophila melanogaster* has at least the excision and photoreactivation repair mechanisms. It remains to be shown whether "recombination-repair" (RUPP and HOWARD-FLANDERS 1968) or postreplication repair (LEHRMANN 1972; BUHL *et al.* 1972) mechanisms are found in Drosophila cells. The excision repair pattern in Drosophila is similar to that which has been reported in human cells (REGAN, TROSKO and CARRIER 1968) while the presence of the photoreactivation repair mechanism makes Drosophila cells similar to the cells of marsupial mammals (COOK 1970) and different from human cells (CLEAVER 1966; TROSKO 1970). These observations should be of some value either in studies of comparative mutagenesis (assuming the repair of non-repair of damaged DNA is somehow related to mutagenesis) or of the mechanism of UV-induced mutagenesis in eukaryotes.

LITERATURE CITED

ALTENBURG, L. S. and E. ALTENBURG, 1957 A comparison of the effect of photoreactivating light on the mutation rates produced by different doses of 2537 A ultraviolet light. Genetics **42**: 358.

BRIDGES, B. A., 1969 Mechanisms of radiation mutagenesis in cellular and subcellular systems. Ann. Rev. Nuclear Sci. **19**: 139–178.

BROWNING, L. S. and E. ALTENBURG, 1962 The proportionality between mutation rate and ultraviolet dose after photoreactivation in Drosophila. Genetics **47**: 361–366.

BUHL, S. N., R. M. STILLMAN, R. B. SETLOW and J. D. REGAN, 1972 DNA chain elongation and joining in normal human and xeroderma pigmentosum cells after ultraviolet irradiation. Biophysical J. **12**: 1183–1191.

CARRIER, W. L. and R. B. SETLOW, 1971 The excision of pyrimidine dimers. pp. 230–237. In: *Methods in Enzymology*. Vol. XXI. Edited by L. GROSSMAN and K. MOLDAVE. Academic Press, New York.

CLEAVER, J. E., 1966 Photoreactivation: A DNA repair mechanism absent from mammalian cells. Biochem. Biophys. Res. Commun. **24**: 569–576.

CLEAVER, J. E. and J. E. TROSKO, 1970 Absence of excision of ultraviolet-induced cyclobutane dimers in xeroderma pigmentosum. Photochem. and Photobiol. **11**: 547–550.

COOK, J. S., 1970 Photoreactivation in Animal Cells. pp. 191–233. In: *Photophysiology*. Vol. **5**. Edited by A. C. GIESE. Academic Press, New York.

DEMEREC, M., A. HOLLAENDER, M. B. HOULAHAN and M. BISHOP, 1942 Effects of monochromatic ultraviolet radiation on *Drosophila melanogaster*. Genetics **27**: 129–140.

GOLDMAN, A. S. and R. B. SETLOW, 1956 The effects of monochromatic ultraviolet light on the egg of Drosophila. Exp. Cell Res. **11**: 146–159.

LEHRMANN, A. R., 1972 Postreplication repair of DNA in ultraviolet-irradiated mammalian cells. J. Mol. Biol. **66**: 319–337.

LIEB, M., 1951 Enhancement of ultraviolet-induced mutation in bacteria by caffeine. Z. Vererbungsl. **92**: 416–429.

LUMB, J. R., A. S. SIDEROPOULOS and D. M. SHANKEL, 1968 Inhibition of dark repair of ultraviolet damage in DNA by caffeine and 8-cholorocaffeine, Kinetics of inhibition. Mol. Gen. Genet. **102**: 108–111.

MUHAMMED, A. and J. E. TROSKO, 1967 A search for photoreactivating enzymes in *Drosophila melanogaster* extracts. Drosophila Inform. Serv. **42**: 82.

PERLITSCH, M. and A. KELNER, 1953 The reduction by reactivating light of the frequency of phenocopies induced by ultraviolet light in *Drosophila melanogaster*. Science **118**: 165–166.

PRUDHOMMEAU, C., 1972 *Irradiation UV des Cellules polaires de L'oeuf chez Drosophila melanogaster. III. Etude de la recombinaison mitotique induite chez le male*. Mut. Res. **14**: 53–64.

REGAN, J. D., J. E. TROSKO and W. L. CARRIER, 1968 Evidence for excision of ultraviolet-induced pyrimidine dimers from the DNA of human cells in vitro. Biophysical J. **8**: 319–325.

RUPP, W. D. and P. HOWARD-FLANDERS, 1968 Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli*, following ultraviolet irradiation. J. Mol. Biol. **31**: 291–304.

SCHNEIDER, I., 1971 Embryonic cell lines of *D. melanogaster*. Drosophila Inform. Serv. **46**: 111.

SETLOW, R. B., 1964 Physical changes and mutagenesis. J. Cellular Comp. Physiol. **64**: Suppl. 1, 51–68.

SETLOW, R. B. and W. L. CARRIER, 1968 The excision of pyrimidine dimers in vivo and in vitro. pp. 134–141. In: *International Conference on Replication and Recombination of Genetic*

Material. Edited by W. J. Peacock and R. D. Brock. Australian Academy of Science, Canberra, Australia.

Setlow, R. B., J. D. Regan, J. German and W. L. Carrier, 1969 Evidence that xeroderma pigmentosum cells do not perform the first step in the repair of ultraviolet damage to their DNA. Proc. Natl. Acad. Sci. **64:** 1035–1041.

Sideropoulos, A. S. and D. M. Shankel, 1968 Mechanisms of caffeine enhancement of mutations induced by sublethal ultraviolet dosages. J. Bacteriol. **96:** 198–204.

Trosko, J. E., 1970 Lack of photoreactivation in human cells grown in vitro. Intern. J. Radiation Biol. **18:** 271–275.

Witkin, E. M., 1958 Post-irradiation metabolism and the timing of ultraviolet-induced mutations in bacteria. Proc. 10th Int. Congress Genet. **1:** 280–299. ——, 1969 UV-induced mutations and DNA repair. Ann. Rev. Genet. **3:** 525–552.