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DIRECT EVIDENCE THAT PYRIMIDINE DIMERS IN DNA RESULT IN NEOPLASTIC TRANSFORMATION*

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SUMMARY

A specific test for the biological role of ultraviolet (UV)-induced pyrimidine dimers in DNA is photoreactivation (PR). Fish contain large amounts of the PR enzyme. Portions of cell suspensions of tissue from various organs of the fish Poecilia formosa were exposed to UV radiation (254 nm), then injected into isogenic recipients. An incident fluence of 20 J/m^2 resulted in 10% of the fish with large granulomas and 100% with thyroid carcinomas. If the irradiated cell suspension was illuminated with PR light before injection, the yields of both types of lesion were reduced ~10-fold. If the PR light was given before the UV exposure, there was no reduction in the numbers of growths. These experiments show that pyrimidine dimers in DNA can lead to neoplastic transformation.

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1. INTRODUCTION

Our interest in ultraviolet (UV) radiation as a carcinogenic agent stems from the large amount of basic photochemical and photobiological information concerning UV damage and its repair and from the fact that human skin cancer is associated with UV radiation (Emmett, 1973). We wish to know whether DNA is the target macromolecule for UV-induced neoplastic transformation, and, if so, what initial molecular changes in DNA result in transformation. The Overview of Chapter X summarizes the evidence indicating that DNA is one of the more important targets for transformation.

Since UV makes many changes in DNA (Setlow and Setlow, 1972), we do not know whether the biologically important photoproducts are cyclobutyl-pyrimidine dimers or one of the many other alterations induced in DNA. The absence of dimer excision in XP cells, for example, is not conclusive evidence that dimers are the important causative agent, because other uninvestigated changes may not be repaired either. One particular repair system — enzymic photoreactivation (PR) — is specific for pyrimidine dimers (see Chap. II). Of all the many products made by UV radiation of DNA, only dimers are affected by PR. The process monomerizes dimers, yielding the original unaltered polynucleotide, but it leaves the other products untouched. Therefore PR can be used as a diagnostic tool. If formation of tumors by UV were prevented by PR, this finding would be evidence that cyclobutyl-pyrimidine dimers are a lesion in DNA that results in neoplastic transformation.

A number of years ago Rogers (1955) showed that if embryonic mouse lung tissue was irradiated in vitro with 254 nm-radiation, it would give rise to adenomas upon transplantation to homologous recipients. Such experiments are attractive since the treatment of cells in vitro permits one to make good physical or chemical dosimetry measurements. However, since mice are deficient in PR activity (Cook, 1970), PR experiments cannot be used to assess the role, if any, of dimers in transformation. Marsupials do contain useful amounts of PR enzyme (Cook, 1970); therefore in 1969 J. S. Cook, J. D. Regan, and R. B. S. designed experiments using marsupials. The experiments were not begun because of the associated housekeeping problems. Meanwhile, a letter in Science (Agranoff et al., 1971) led J. D. Regan to suggest the use of clones of fish as an experimental system. Fish cells contain large amounts of PR enzyme (Cook, 1970). Furthermore, the fish we use, Poecilia formosa, may be grown in clones, allowing cells from one animal to be easily treated in vitro and transplanted successfully to another (Kallman, 1962).

2. EXPERIMENTAL METHODS

We used several clones of the naturally occurring gynogenetic (nonsexually reproducing) fish P. formosa. The offspring of the fish are white and identical, and since the females are activated by the males of another species (black), the transmission of any male information is easily detected by the mottled skin color (Hart et al., 1974). The general experimental design is shown in Fig. 1. Tissues

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were homogenized to yield cell clumps (3-8 cells per clump), the clumps were treated as indicated in Fig. 1, and 20 μl containing 3-5 X 10^5 cells was injected into the abdominal cavity of isogenic recipients (~3 to 4 months in age). In these experiments several tissues were homogenized together. Hence injected cells consisted of a mixture of liver, heart, and thyroid cells. The latter tissue represented ~40% of the injected cells. Fish were killed 6 to 9 months after the injection of cells and fixed in Bouin's solution. Tumors were scored by gross pathology and by the appearance of stained histological sections. We are deeply indebted to John Harshbarger of the Registry of Tumors in Lower Animals, Smithsonian Institution, Washington, D.C., for the histological analyses. It was he who first noticed that the fish injected with irradiated cells had thyroid carcinomas. His histological conclusions were confirmed by C. J. Dawe of the National Cancer Institute.

3. RESULTS

Our results obtained by injecting treated liver cells into the back muscle of fish have been described briefly (Hart and Setlow, 1973). After an incident UV fluence of 20 J/m^2, ~10% of the fish had large tumors at the site of injection (gross pathology and histological examination by B. Koesner, Veterinary Pathobiology Department, Ohio State University). Exposure of the cells to PR illumination

after UV resulted in a large decrease in the numbers of tumors — a dose reduction factor of ~0.8. PR illumination by itself had little effect. Because such highly localized tumors were not suitable for histological examination, we turned to experiments in which treated cells were injected into the abdominal cavity.

The initial attempts using cells injected into the abdominal cavity were not useful because of the presence of tuberculosis organisms in many of the fish. Many of the experimental animals contracted the disease and many had granulomas that might have been bacterially induced. After assuring that the colony was clean, we resumed the experiments. Most of the fish injecred with UV-irradiated cells had large numbers of noninfectious granulomas and, in addition, had thyroid carcinomas which were invasive into both soft ard hard tissue. Thyroid carcinomas were present in 100% of the fish injected with 5×10^5 cells exposed to an average incident fluence of 10-20 J/m^2. Smaller or larger fluences gave fewer tumors. Table 1 shows the effect of PR illumination before and after the UV exposure. (T-1) Note thar PR after UV irradiation results in a very large decrease in the number of thyroid carcinomas, whereas illumination before UV does not. A similar finding is observed for the granulomas. Thus these data implicate pyrimidine dimers in DNA as the initial change that results in neoplastic transformation.

Treatment of cells with N-acetoxy-AAF before injection resulted in the appearance of thyroid carcinomas in a large percentage of the fish, as shown in Table 2. (T-2)

4. CONCLUSIONS

The experiments described here show that single exposures of fish cells to UV radiation result in neoplastic transformation, and that if the UV irradiation is followed by PR illumination, many fewer transformants arise. Our background knowledge of photochemistry and photobiology summarized in this volume leads us to conclude that the initial change in macromolecules resulting from the UV irradiation has been in DNA and, moreover, that the particular change in DNA has been the formation of pyrimidine dimers. The data, of course, say nothing about the mechanism(s) by which such changes are converted into neoplastic transformations.

It is instructive to estimate the probability of a transformation per dimer in cellular DNA. We estimate (from data in this paper and also unpublished) that an average incident fluence of 5 J/m^2 will result in approximately one transformed cell per 10^5 thyroid cells. This fluence converts $\sim 0.02\%$ of the thymines to thymine-containing dimers, which corresponds to ~ 1 dimer/10^7 daltons of DNA. Since there are about 10^{12} daltons of DNA per cell, there are 10^5 dimers/cell at 5 J/m^2. Hence the probability of o random dimer resulting in a neoplastic transformation is $\sim 10^{-10}$.

Studies on the magnitude of DNA repair following treatment of normal human cells with UV and with N-acetoxy-AAF indicated that 10^{-5} M AAF was approximately equivalent to 5 J/m^2 (Setlow and Regan, 1972). In the experiments described here, 10^{-4} M AAF was equivalent to ~ 10 J/m^2. This comparison between measurements of repair in one species (human) and transformation in another (fish) may be speculative, but the agreement isn't bad. We take these data as reinforcing the idea that damage to DNA is important tochemical carcinogenesis.

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Table 1

The effects of PR illumination[a] on UV-irradiated[b] cells[c] of P. formosa

Treatment	Fraction of fish with granulomas	Granulomas per fish	Fraction of fish with thyroid carcinoma by	
			gross pathology	histology
UV	51/63	1.8	34/34	29/29
2.5 min PR + UV	49/57	2.0	26/26	22/22
5.0 min PR + UV	37/42	1.9	48/50	22/23
UV + 5.0 min PR	15/43	0.4	1/43	0/6

[a] 700 $J/m^2/min$ (320-420 nm).

[b] 12 J/m^2 average incident fluence (254 nm).

[c] $\sim 3 \times 10^5$ cells injected per fish.

Table 2

The effect of N-acetoxy-AAF[a] on cells[b] of P. formosa

Concentration	Fraction of fish with thyroid carcinoma by	
	gross pathology	histology
0	0/50	—
10^{-5} M	14/50	—
10^{-4} M	45/51	10/10

[a] Treatment for 1 hr at the indicated concentration.

[b] $\sim 5 \times 10^5$ cells injected per fish.

FIGURE LEGEND

Fig. 1. Outline of an experiment to determine whether pyrimidine dimers in the DNA of irradiated cells result in neoplastic transformation. If they do, then there should be very few tumors in those fish injected with cells treated with UV plus PR compared with UV alone or compared with PR plus UV. The no-treatment and PR samples are controls.

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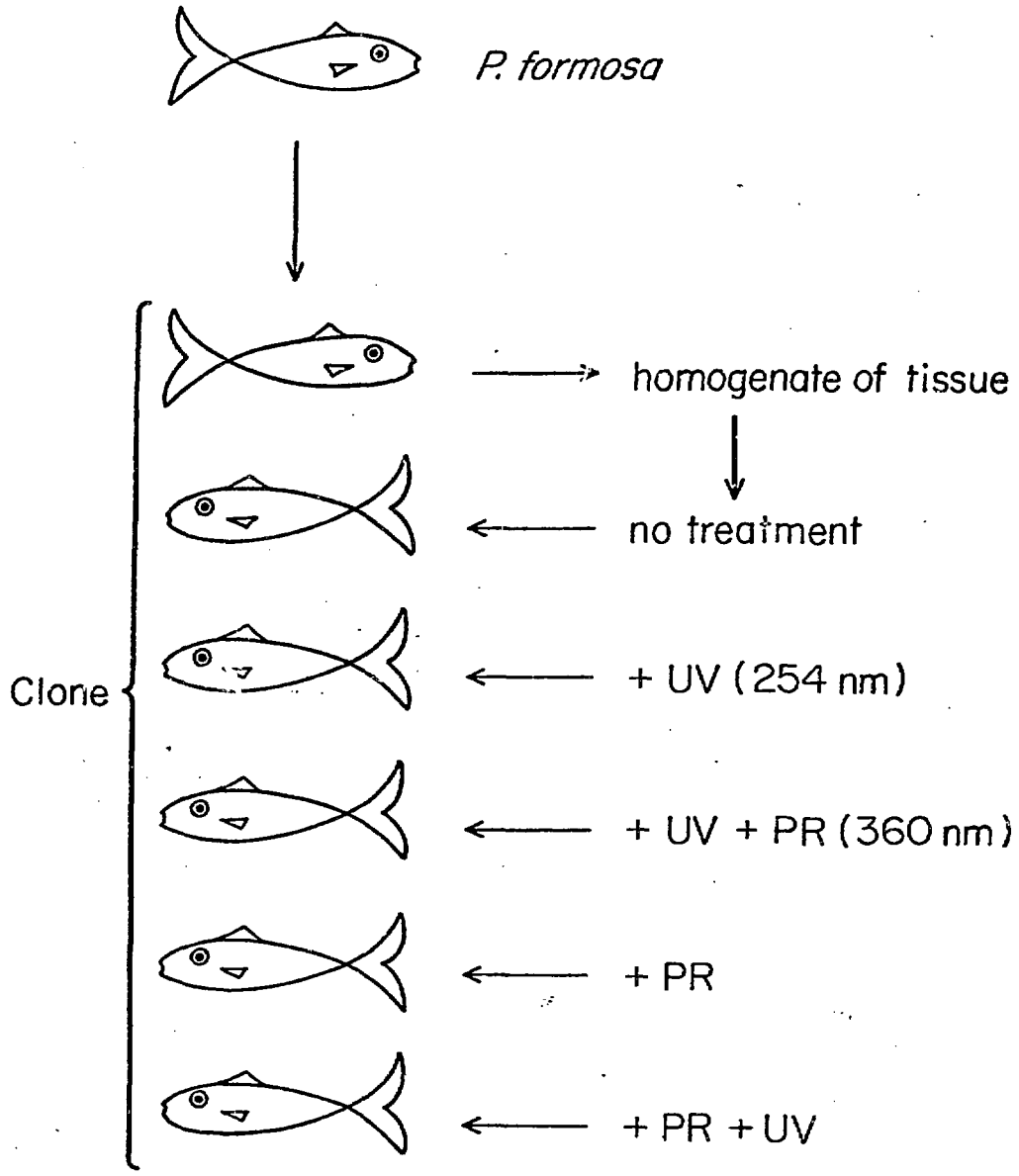
DO PYRIMIDINE DIMERS IN DNA RESULT IN TUMORS?



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