

Detection of Laser-UV Microirradiation-induced DNA Photolesions by Immunofluorescent Staining

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Summary. A low-power laser-UV microbeam of wavelength 257 nm was used for microirradiation of a small part of the nucleus of Chinese hamster cells. Following fixation in interphase or in the subsequent metaphase indirect immunofluorescent staining was performed with antiserum to photoproducts of DNA treated with far UV light.

The results show that antibodies specific for UV-irradiated DNA can be used for a direct detection of laser-UV microirradiation-induced DNA photolesions. The potential usefulness of this method for investigation of the spatial arrangement of chromosomes in the interphase nucleus is discussed.

Introduction

Currently available methods of detecting the distribution of DNA photolesions induced by UV microirradiation in mammalian cells (Moreno, 1971; T. Cremer et al., 1979; Zorn et al., 1979) make use of unscheduled DNA synthesis (UDS).

This technique, however, allows only detection of the sites of excision repair, and not of the DNA photolesions themselves. In this contribution a new approach is described, which allows direct demonstration of DNA photolesions in individual UV-microirradiated cells by use of antibodies specific to UV-irradiated DNA (Levine et al., 1966; Fukuda et al., 1976). Such antibodies have been used to study the potency of excision repair in single cells following whole-cell

irradiation (Lucas, 1972; Fukuda et al., 1976; Cornelis et al., 1977).

Material and Methods

Laser-UV Microbeam

A laser-UV microbeam of wavelength 257 nm was used, as described in detail elsewhere (Cremer et al., 1974, 1976). Briefly, a coherent UV beam of wavelength 257 nm, obtained by frequency doubling of an argon ion laser beam of wavelength 514 nm, is focused into the object plane of the irradiation microscope by means of a quartz microscope objective. Here, we used a Zeiss Ultrafluor 32×/0.40 Ph Glyz giving a minimum spot diameter of approx. 1 μm. The irradiation objective is used simultaneously for observation of the target cells in phase contrast. Aiming is performed by means of cross-hairs in the ocular.

Cell Material

Fibroblastoid Chinese hamster cells (CHL) were obtained from lung tissue of a 3-week-old animal and grown under standard conditions (Zorn et al., 1976). Cells of passages 10–15 were used for experiments. In some cases experiments were performed with a V79 subline of the Chinese hamster (Cremer et al., 1976).

Preparation of Antiserum to UV-irradiated DNA

Antibodies to UV-irradiated DNA (mainly pyrimidine dimers) were prepared as described previously (Fukuda et al., 1976). Briefly, denatured calf thymus DNA was irradiated with UV light of wavelength 254 nm and complexed with methylated bovine serum albumin (MBSA). The DNA-MBSA complex was used as an antigen to produce antibodies in rabbits. Immunodiffusion reactions showed that the antibodies produced in this way bind specifically to UV-irradiated DNA.

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Irradiation and Fixation

CHL cells synchronized by mitotic selection or asynchronously growing cells were inoculated onto glass slides in petri dishes. Before the inoculation, a number of squares were marked on the glass slides by means of a diamond pencil. Cells situated in these squares ('experimental field') were selected for microirradiation. The cells outside the experimental field were not irradiated and served as controls. In this way it was possible to relocate irradiated and unirradiated cells.

For microirradiation, the petri dishes containing the glass slides with the inoculated cells were transferred to a special irradiation chamber (Cremer et al., 1976). Then the cells were subjected to laser-UV microirradiation at a small part of the nucleus.

Following microirradiation two schedules were used: In Schedule I the cells were fixed with methanol immediately after irradiation; in Schedule II, the cells were grown for 6–19 h, and an *in situ* chromosome preparation (Zorn et al., 1976) was made.

In some cases, whole-cell irradiation was performed by means of a germicidal lamp emitting essentially UV light of wavelength 254 nm. These cells were fixed according to Schedule I.

Immunofluorescent Staining

After fixation (Schedule I, II), cells were first incubated with antiserum, washed with phosphate-buffer saline, and conjugated with fluorescein isothiocyanate (FITC)-labeled anti-rabbit immunoglobulin antibodies (indirect immunofluorescent technique).

Results

Fig. 1 shows a result of antibody staining in CHL cells fixed immediately following microirradiation (Schedule I). Small dots of strong fluorescence were detected in the nuclei of microirradiated cells. The fluorescence was localized to a small part of the nucleus. The strength of this localized fluorescence increased with incident UV energy.

No fluorescence above background was observed in unirradiated control cells. Furthermore, this type of localized fluorescence was not detected in cells exposed to whole-cell UV irradiation. We conclude that the localized fluorescence is due to binding of antibodies to DNA photolesions produced by the laser-UV microirradiation in a small part of the nucleus. The sensitivity of the antibody technique allowed detection of localized fluorescence at an incident UV energy as low as 0.3 nJ, this energy being compatible with cell proliferation (Cremer et al., 1976).

In Fig. 2a an antibody-stained metaphase plate obtained from a V 79 cell microirradiated at one pole of the nucleus in the preceding interphase is shown (Schedule II). Fluorescence labeling was found to be restricted to a few chromosome segments only. Specific

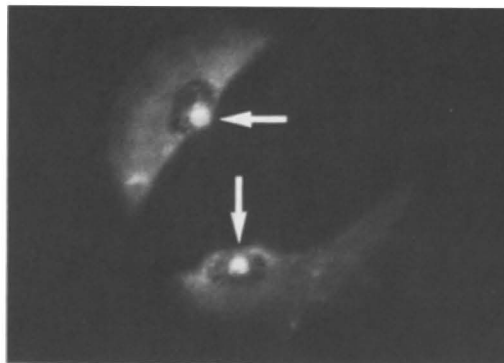


Fig. 1. Antibody staining of a CHL cell fixed immediately after laser UV microirradiation of a small part of the nucleus (incident UV energy 0.3 nJ) of cells synchronized in G₁. The arrows indicate the site of specific fluorescence

fluorescence was not found in metaphase plates from unirradiated cells.

The uneven distribution of antibody binding on the fluorescence-labeled chromosomes (Fig. 2b) may reflect local differences in thymidine content resulting in different amounts of thymidine dimers (Schreck et al., 1974) and/or different repair capacity along the chromosomes (Berliner et al., 1976; Johnson and Sperling, 1978).

Discussion

The results show that antibodies specific for UV-irradiated DNA can be used for a direct detection of laser-UV microirradiation-induced DNA photolesions. The spot size obtained by antibody staining in Chinese hamster nuclei fixed immediately after microirradiation was found to be similar to that observed in autoradiographs of cells after microirradiation treatment in G₁ and pulse-labeling with ³H-thymidine (T. Cremer et al., 1979; Zorn et al., 1979).

Specific antibody binding was also observed following laser-UV microirradiation of the interphase nucleus of V 79 cells and indirect immunofluorescent staining in the following metaphase (Fig. 2). This means that the amount of nonexcised DNA photolesions was still large enough to be detectable by antibody staining. The finding that the fluorescence labeling was restricted to a few chromosomes is in keeping with the outcome of previous experiments with CHL cells (C. Cremer et al., 1979; Zorn et al., 1979):

In these experiments, the cell nucleus was microirradiated in G₁ and pulse-labeled with ³H-thymidine. UDS served as a label to follow the microirradiated chromatin from interphase to metaphase. UDS label

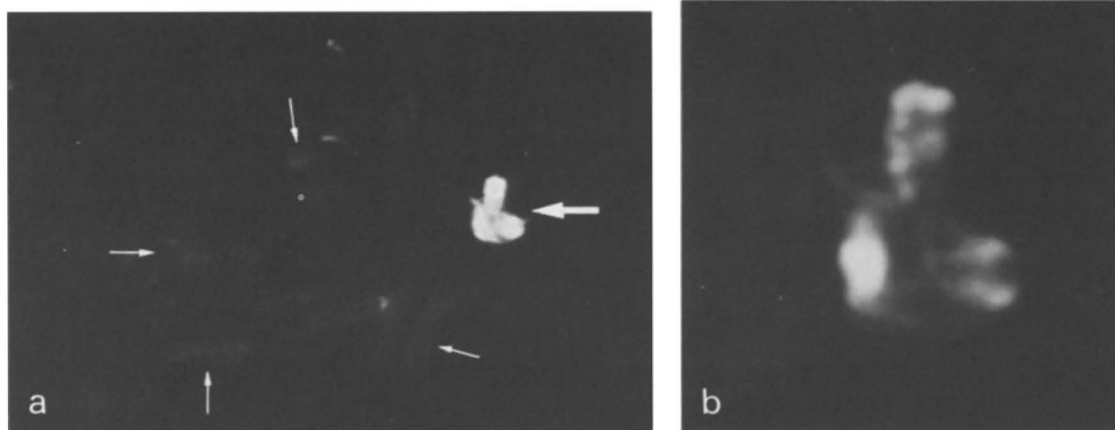


Fig. 2. **a** and **b** Microphotograph of an antibody-stained metaphase plate from a V79 cell following laser-UV microirradiation of a small part of the nucleus (incident UV energy 0.3 nJ) in the preceding interphase. Chromosome preparation was performed 9 h after irradiation.

a Micrograph with a long exposure time: The *large arrow* (→) indicates two brightly fluorescing chromosomes lying adjacent to each other at the periphery of the metaphase plate (labeled chromosomes); the *small arrows* (→) indicate unlabeled chromosomes, which are visible due to faint background fluorescence as observed in metaphase plates obtained from nonirradiated cells.

b Micrograph of the labeled chromosomes shown in **a** (*large arrow*) but with a short exposure time. Note the uneven distribution of antibody binding

was found to be concentrated to a few chromosomes lying fairly close to each other in one part of the metaphase plate.

We suggest that both methods can be used to investigate the spatial arrangement of interphase chromosomes (Comings, 1968; Vogel and Schroeder, 1974; Stack et al., 1977; Comings, 1979) according to the following experimental rationale: Cells are microirradiated at a small part of the nucleus. The microirradiated chromatin is detected in the chromosomes of the following metaphase either by unscheduled DNA synthesis or directly by antibodies specific for DNA photolesions. The labeled chromosome segments must have been situated close to each other in the preceding interphase at the time and site of irradiation.

Compared with the UDS protocol, the antibody technique has several important advantages: (1) No confusion is possible with replication patterns of semiconservative DNA synthesis; (2) all stages of the interphase cycle can be investigated; and (3) the antibody technique can be used in a wider variety of cell strains, especially in those with low levels of repair DNA synthesis (Cleaver and Trosko, 1969; Cleaver, 1974). The method can also be applied to cells of normal excision repair capacity, because even a relatively long time (24–48 h) after irradiation a substantial amount of dimers is retained (Cleaver et al., 1972; Amacher et al., 1977; Inoue and Takebe, 1978).

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