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RESEARCH NOTE

LASER-UV-MICROIRRADIATION OF CHINESE HAMSTER CELLS: THE INFLUENCE OF THE DISTRIBUTION OF PHOTOLESIONS ON UNSCHEDULED DNA SYNTHESIS

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Abstract—Fibroblastoid Chinese hamster cells synchronized by mitotic selection were microirradiated in G1, using a low power laser-UV-microbeam (λ = 257 nm). The incident energy was either concentrated on a small part of the nucleus (mode I) or distributed over the whole nucleus (mode II). Using the same incident UV energy, the local UV fluences were estimated to differ by two orders of magnitude. Following microirradiation the cells were incubated with [^{3}H]-thymidine for 2 h and thereafter processed for autoradiography. Silver grains were concentrated over the microirradiated part after mode I and distributed over the whole nucleus after mode II irradiation. To quantify the amount of unscheduled DNA synthesis, the number of grains per nucleus was determined. It increased with the total incident energy, but was not or only slightly affected by the mode of microirradiation, if appropriate autoradiographic conditions were used. The findings suggest that within the investigated range of energy densities (2.7–1000 J/m^2), the total amount of unscheduled DNA synthesis depends on the total number of pyrimidine dimers but not on their distribution in nuclear DNA.

INTRODUCTION

Since the discovery of unscheduled DNA synthesis (UDS)† in mammalian cells (Rasmussen and Painter, 1964), extensive literature has been accumulated concerning UDS following UV-irradiation of whole cells. Little is known, however, on the influence of different distributions of DNA photolesions.

Microirradiaton (for review, see Moreno, 1969; Berns, 1974) provides an excellent tool to investigate the effect of such different spatial distributions of a given amount of UV light within the cell nucleus. So far, however, there is only one report where this possibility has been exploited (Moreno and Salet, 1974). Using a conventional UV-microbeam of wavelength 254 nm and asynchronously growing human KB cells, these authors have concluded that wherever the photons are localized within the nucleoplasm, the amount of UDS depends only on the total number of incident photons and not on the area of irradiation. This conclusion was based on a quantitative evaluation of autoradiographs from KB nuclei which were microirradiated at one, two or four sites with energy densities of 100 and 200 J/m^2, respectively, and subsequently pulselabeled with [^{3}H]-thymidine. Since it has been reported (Lehmann and Kirk-Bell, 1972) that the level of excision repair may drastically depend on the energy density applied, it is conceivable that the distribution of incident photons affects UDS considerably when a larger range of energy densities is used. Furthermore, one would like to know whether the effect reported by Moreno and Salet is a general feature of mammalian cells or a characteristic of the transformed cells used.

In the present investigation, we studied the influence of various distributions of UV-induced DNA photolesions on UDS in fibroblastoid Chinese hamster cells of essentially normal diploid status synchronized in G1. In further contrast to Moreno and Salet, different distributions of DNA photolesions were realized by either concentrating the UV-quanta to a randomly selected part of the nucleoplasm (mode I) or distributing them approximately over the whole nucleus (mode II). Thus the energy density in the microirradiated part of the nucleus differed for two orders of magnitude in mode I and mode II experiments. A total range of energy densities between 2.7 and 1000 J/m^2 was investigated. Finally it should also be noted that in the experiments of Moreno and Salet, the 'large area' distribution of incident photons was very inhomogeneous. Even when four sites of the nucleus were microirradiated, still only half of the

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† *Abbreviations*: CHL, Chinese hamster cells; FUDS, factors which limit rate of UDS; and UDS, unscheduled DNA synthesis.

nuclear area was exposed to UV light while the other half was not. It is, therefore, difficult to compare their data with results obtained by total cell irradiation where the distributions of incident photons is homogeneous over the whole nuclear area. In mode 11 experiments we have achieved a rather homogeneous irradiation of the major part of the nucleus, while most of the cytoplasm was still excluded from irradiation. Thus, we were able to compare an extremely inhomogeneous distribution of incident photons (mode 1) and a rather homogeneous one (mode 11). Our study fully confirms the conclusion of Moreno and Salet even under these extreme experimental conditions.

MATERIALS AND METHODS

The laser-UV-microbeam of wavelength 257 nm has been described in detail elsewhere (Cremer *et al.*, 1974). Briefly, a coherent continuous wave UV-beam of wavelength 257.25 nm is focused by a Zeiss ultrafluar objective. This objective is simultaneously used for both microirradiation and phase contrast observation. By changing the position of an adapting lens, different diameters of the irradiation field in the object plane can be adjusted. Thus, different distributions of a given amount of UV-quanta are obtained. Due to the low power incident at the cell surface (8×10^{-9} W), no 'laser specific' effects are expected to occur (Cremer *et al.*, 1976).

Cell material. Fibroblastoid Chinese hamster cells (CHL) were obtained from lung tissue of a 3-wk-old female and grown under standard conditions (Zorn *et al.*, 1976). In this investigation, cells of passage numbers 10–15 were used. At this passage level the majority of cells had still maintained a normal diploid status as shown by Q-banding analysis. Whole cell irradiation experiments at wavelength 254 nm (Cremer *et al.*, 1978) and UV-microirradiation experiments (Zorn *et al.*, 1979) indicated that these cells are capable of UDS.

Microirradiation and posttreatment. Cells synchronized by mitotic selection (Zorn *et al.*, 1979) were inoculated into 6 cm plastic petri dishes (Nunc/Denmark) and kept in medium with 0.5% fetal calf serum until microirradiation was performed (12–14 h later). Under these conditions, a large majority of the cells was still in G1 as indicated by pulse labeling with [^{3}H]-thymidine (see Results). Prior to inoculation, a number of small squares (0.5 × 0.5 mm each) was marked in the bottom of the dishes by means of scalpel cuts. Only cells situated in these squares ('experimental field') were selected for microirradiation and could easily be relocated for further observation. Cells outside the experimental field served for control purposes. For microirradiation, the petri dishes were placed into a special irradiation chamber (Cremer *et al.*, 1976). To all cells of clear interphase morphology lying in a given square of the experimental field, the same microirradiation treatment was applied.

Microirradiation of the nucleus was performed in two ways: (i) microirradiation mode 1 at a randomly selected site with an irradiation field of approximately 1 μm diameter in the object plane; (ii) microirradiation mode 11 with an irradiation field of approximately 12 μm diameter (corresponding to the mean nuclear diameter).

In a given petri dish, both microirradiation modes were used. The incident UV-energy delivered to a microirradiated cell ranged from 0.27 nJ to 2 nJ. By dividing these values by the area of the respective irradiation fields, the local energy density was estimated to be 300–2000 J/m^2 in case of mode 1 and 2.7–18 J/m^2 in case of mode 11 irradiation. Following microirradiation, the cells were incubated with [^{3}H]-thymidine (10 μCi/m/, 40 Ci/mmol) for 2 h and fixed with glutaraldehyde. Autoradiography was performed using standard procedures (Moreno, 1971; Zorn, 1978). The preparations were stained with acetic orcein.

RESULTS

Figure 1a shows an autoradiograph of a cell after mode 1 irradiation. The silver grains indicating the site of UDS are concentrated to a small part of the nucleus. Videotape recordings confirmed that the site of irradiation and the site of UDS are identical (Zorn, 1978). In Fig. 1b, a cell is shown after mode 11 irradiation. In this case, the silver grains were distributed over the whole nucleus. In most cases, the cells could easily be distinguished from the small percentage of S-phase cells present which exhibited much larger grain numbers, as established from unirradiated controls. In the experiments described here, in the mean only 2% of the cells showed S-phase labeling and were excluded from further evaluation.

To quantify the *spatial* extent of UDS following mode 1 irradiation, the nuclear area F covered by silver grains was determined from camera lucida drawings obtained from microscopic observations. F covered 2–10% of the total nuclear area, the mean value being approximately 3%. This confirmed that the spatial distribution of UDS was indeed very different for the two modes of microirradiation.

Table 1. Influence of the distribution of photolesions on UDS (grain numbers in G1-nuclei)*

Expt No.	E_{inc} (nJ)	N_I	N_{II}	f
1a	0.27	26.8 ± 3.4 (N = 85)	29.5 ± 4.4 (N = 43)	1.1
1b	1.0	46.4 ± 3.8 (N = 81)	51.1 ± 5.4 (N = 41)	1.1
2a	0.53	22.8 ± 1.2 (N = 106)	30.6 ± 4.7 (N = 37)	1.3
2b	1.0	30.4 ± 1.7 (N = 91)	30.9 ± 4.5 (N = 30)	1.0

*CHL-cells were UV-microirradiated (λ = 257 nm) in G1. The incident energy was either concentrated on a small part of the nucleus (mode 1) or distributed over approximately the whole nucleus (mode 11). Following microirradiation, the cells were pulse labeled with [^{3}H]-thymidine. In autoradiographs, the number of silver grain per nucleus was counted. All cells microirradiated in Experiment 1a and 1b were situated in the same petri dish A; all cells microirradiated in Experiment 2a and 2b were situated in the same petri dish B.

E_{inc}: UV-energy incident at the cell surface.

N_I, N_{II}: grain number per nucleus (mean + SDM) corrected for background (mean of grain number in unirradiated cells). The number N of nuclei evaluated is given in parentheses.

N_I: microirradiation mode 1.

N_{II}: microirradiation mode 11.

f: = N_{II}/N_I (ratio of mean values).

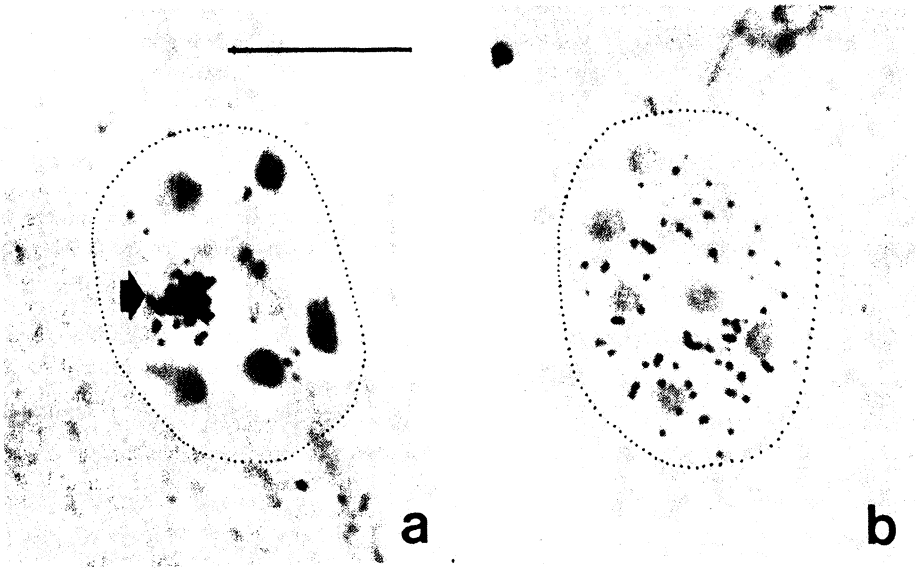

Figure 1. Autoradiographs following laser-UV-microirradiation of the nucleus of CHL-cells in G1 (incident UV-energy 2 nJ). (a) mode 1 (microirradiation of a small part of the nucleus). Arrow indicates site of unscheduled DNA synthesis. (b) mode 11 (microirradiation of approximately the whole nucleus). The nuclear edge is marked by a dotted line. Bar: 10 μm.

To quantify the *total amount* of UDS per nucleus induced by the two modes of irradiation, the number of silver grains per G1-nucleus was determined.

In Table 1, the results of two series of experiments are summarized. In Experiment 1, the grain numbers were found to be the same (within statistical limits) after both modes of microirradiation. An average of $N_1 = 26.8$ silver grains per nucleus was determined in G1-cells following mode 1 irradiation with an incident UV-energy $E_{inc} = 0.27$ nJ (Expt 1a). When the same incident UV-energy was distributed approximately over the whole nucleus by mode 11 irradiation, an average of $N_{11} = 29.5$ grains per nucleus was detected. The value of $f = N_{11}/N_1$ was found to be 1.1. The amount of UDS increased with E_{inc}. After irradiation with $E_{inc} = 1$ nJ, in the mean 46.4 grains after mode 1 and 51.1 grains after mode 11 irradiation were obtained, the f-value again being 1.1 (Expt 1b). In Experiment 2, the grain numbers were somewhat lower due to slightly modified autoradiographic conditions. The principal outcome, however, is the same as obtained in Experiment 1.

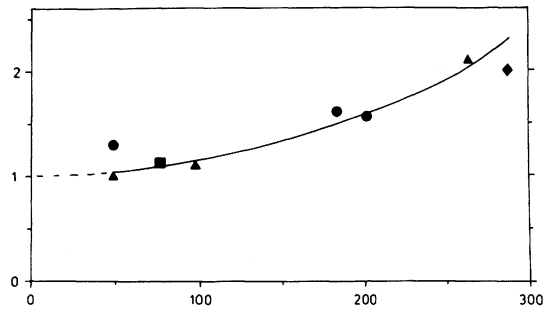

Figure 2. Influence of the grain number on the apparent f-value. CHL-cells were microirradiated and pulse labeled with [^{3}H]-thymidine as described (see information in Table 1). Petri dishes with identical irradiation treatment (e.g. incident UV-energy $E_{inc} = 0.53$ nJ mode 1 and mode 11 irradiation respectively) were processed for autoradiography, using different exposure times (1–2 wk). In autoradiographs, the number of silver grains per nucleus was determined, and $f = N_{II}/N_I$ was calculated (see information in Table 1). On the average, for each f-value the grain numbers in 100 nuclei were counted. Ordinate: $f = N_{II}/N_I$. Abscissa: total grain number per nucleus following mode 11 irradiation (no correction for background). ■: $E_{inc} = 0.27$ nJ, ●: $E_{inc} = 0.53$ nJ, ▲: $E_{inc} = 1.0$ nJ, ◆: $E_{inc} = 2.0$ nJ.

To elucidate the bias introduced by the much larger grain densities in case of mode 1 irradiation, a series of experiments was made, using identical irradiation treatment but different autoradiographic conditions (exposure times). By this, different grain numbers were obtained. Figure 2 shows that the f-value observed indeed depends on the grain number per nucleus. As expected, the f-value increases with increasing N_{11} due to the fact that at high grain densities (mode 1) the total number of grains (N_1) is underestimated. The extrapolation of f to very low grain numbers, however, results in a f-value close to one.

DISCUSSION

The results presented here show that the *spatial distribution* of UDS induced by laser-UV-microirradiation of CHL-cells is very different whether a given amount of UV-quanta is concentrated to a small part of the nucleus (mode 1) or distributed over the whole nucleus (mode 11).

The *total amount* of UDS per nucleus, however, was found to be rather similar for the two modes of microirradiation, the energy densities differing from approximately 2.7 J/m^2 to approximately 1000 J/m^2.

It is known that within this range of energy densities there is an approximately linear increase with UV-fluence of the percentage of pyrimidine dimers in the DNA of Chinese hamster cells (Steward and Humphrey, 1966). This indicates that under these conditions, the total number of pyrimidine dimers in the mean depends on the total incident energy but not on its distribution in the nucleus. Therefore, our findings suggest that within this range the amount of UDS depends on the total number of pyrimidine dimers but not on their distribution in nuclear DNA. As a possible interpretation we suggest that the factors (FUDS) which limit the rate of UDS under the conditions investigated represent a pool available at any part of the nucleus. They are able to move freely ('pool') within a short time compared with the labeling period (2 h). In the 'pool' model, saturation of the excision system occurs only if the *total* number of DNA photolesions per nucleus exceeds a certain level. It should be noticed that in spite of freely moving FUDS-factors, the repair capability may be different in different parts of the nucleus (Harris *et al.*, 1974; Berliner *et al.*, 1975; Smerdon *et al.*, 1978), e.g. due to different 'masks' of nucleoprotein (Wilkins and Hart, 1974) which may interfere with the action of the factors. However, since the sites of microirradiation mode 1 in the nucleus were selected randomly, such differences in repair capability did not affect the ratio ($f = N_{11}/N_1$) of the mean grain numbers. It would be interesting to know whether the independence of the total amount of UDS on the spatial distribution of the incident photons over a wide range of energy densities observed in the CHL cells used here is a general feature of mammalian cells. If so, then from a biological point of view, the total incident energy delivered per cell nucleus might be a more appropriate parameter to describe the capability of cells to perform UDS than the energy density (UV-fluence) usually used.

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