SISTER CHROMATID EXCHANGES

25 Years of Experimental Research

Part A
The Nature of SCEs

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SISTER CHROMATID EXCHANGE (SCE) INDUCED BY LASER-UV-MICROIRRADIATION: CORRELATION BETWEEN THE DISTRIBUTION OF PHOTOLESIONS AND THE DISTRIBUTION OF SCEs

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SUMMARY

Small, medium, and large nuclear areas comprising approximately 5, 30, or 80% of the total area of the interphase nuclei of Chinese hamster cells (M3-1) cultivated in vitro were irradiated with a laser-UV-microbeam of wavelength 257 nm. The DNA of the cells was substituted with 5-bromodeoxyuridine (BrdUrd) for 1 cell cycle in one set of experiments. After microirradiation the cells were grown for a second cycle in medium without BrdUrd (protocol A). In a second set, cells with nonsubstituted DNA were microirradiated and grown for 2 additional cycles, the first in the presence, the second in the absence of BrdUrd (protocol B). In situ chromosome preparation and differential chromatid staining was subsequently performed.

The induction of sister chromatid exchanges (SCEs) was found to be dependent on both the ultraviolet (UV) dose and the spatial distribution of the UV energy within the cell nucleus. Following both

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protocols the average number of chromosomes with SCEs was significantly higher after microirradiation of a large nuclear area as compared to microirradiation of a small nuclear area. In the latter case, multiple SCEs were noted on individual chromosome arms at the first postirradiation mitosis (protocol A). In other cells, especially at higher doses, protocol A resulted in shattering of a few closely neighbored chromosomes which were surrounded by intact ones with normal SCE levels. Microirradiation of medium-sized nuclear areas produced high levels of SCEs over a number of chromosomes which still appeared spatially related in a part of the metaphase spread. Finally, high SCE levels could be observed over most or all chromosomes when a large nuclear area (up to 100%) was exposed to the microbeam. Following protocol B the increase of SCEs was much less pronounced. Microirradiation of a small part of the cytoplasm in addition to the nuclei did not induce SCEs. Our results support the concept (i) that interphase chromosomes occupy distinct nuclear domains and indicate (ii) that the induction of SCEs by UV light is restricted to microirradiated chromatin.

INTRODUCTION

Many models have been proposed to elucidate the basic mechanism underlying the formation of SCEs (1-6). There appears to be general agreement that directly or indirectly induced DNA lesions are responsible for SCE induction. The exact relationship, however, between DNA damage, DNA repair, and SCE formation remains still largely unknown (7,8). One of the basic problems associated with mutagen-induced SCE formation is whether SCEs are always formed at (or near) the sites of DNA lesions or whether indirect effects exist by which SCEs are induced in undamaged replicons or replicon clusters in a damaged cell. A possible role of indirect effects has recently been suggested by Painter (5), who proposed that all factors which disrupt the timing of DNA replication can promote the formation of SCEs. Experimental evidence (9-11) indicates that following the application of chemical mutagens, SCEs may be induced not only by factors inside the DNA (damaged template) but also outside the DNA (altered replication machinery). Circumstantial evidence obtained by whole cell UV-irradiation experiments (12-15) indicates that the formation of SCEs depends on the presence of DNA photolesions in a dose-dependent way. For this process, replication of damaged DNA is essential (16). However, it seems possible that DNA photolesions in replicon clusters contained in one part of the nucleus might influence DNA replication in replicons at other sites of the nucleus as well. Circumstantial evidence for such an assumption has been provided by UV-microbeam experiments. Autoradiographs obtained after partial irradiation of nuclei in S phase suggested a considerable reduction of ^{3}H-thymidine incorporated not only within the microirradiated nuclear segment but also in the nonirradiated part of the nucleus (Ref. 17 and our unpublished data). Another example for an indirect effect of UV-irradiation is generalized chromosome shattering (GCS), i.e., all chromosomes of a mitotic cell appear fragmented

or pulverized (18). GCS can be induced with high frequencies both by microirradiation of the total nuclear area and by exposing a small nuclear area (less than 5%) to the same incident UV dose (19-21). To explain these results, a model was proposed (20,22) which takes into account the S phase dependence of this effect. The model predicts that under certain conditions, DNA replication is altered not only in replicons bearing DNA photolesions but in all replicons of a nucleus, even if they do not contain any photolesions.

In this paper, we attempt to answer the question of whether such indirect effects are also important for the induction of SCEs by UV. In addressing this problem, we have formulated 2 alternative hypotheses:

(i) SCEs are formed exclusively at or near the sites of DNA photolesions.

By "near" we mean that SCE formation would take place within the same replicon cluster as the replicon bearing the photolesions.

(ii) The number of SCEs depends on the total number of DNA photolesions, but the sites of SCEs and the sites of photolesions are not necessarily correlated. According to the second hypothesis, some SCEs may be formed by an indirect, S phase dependent effect in undamaged replicon clusters which are situated remotely from damaged ones.

The following rationale has been used to discriminate between these 2 hypotheses. UV-microirradiation provides a tool to realize strongly different distributions of DNA photolesions without changing the total incident energy (23). Small, medium, or large nuclear areas of Chinese hamster cells comprising approximately 5, 30, or 80% of the total area of an average sized nucleus, respectively, were irradiated with a laser-UV-microbeam of wavelength 257 nm (24, 25). Depending on the actual experimental protocol, sister chromatid differentiation was achieved at the first or second postirradiation mitosis. Depending on the mechanism of SCE induction, different predictions concerning the distribution of microirradiation induced SCEs can be made. These predictions are based on our findings that interphase chromosomes in Chinese hamster cells are organized in distinct nuclear territories or domains during the entire cell cycle (26-28). If approximately the entire nuclear area is exposed to a sufficiently large UV-dose, then more or less all chromosomes are expected to exhibit SCE levels above control levels for both hypotheses (i) and (ii). However, if the same UV-dose is selectively applied to a small part of the nucleus, a decision between the 2 hypotheses should become possible. If hypothesis (i) is valid, we should find an increased level of SCEs restricted to the few microirradiated chromosomes. For hypothesis (ii) we would predict that the different distributions of DNA photolesions would have little if any effect on the distribution of SCEs in the metaphase chromosomes.

It has been shown (29) that the types of DNA photolesions produced by ultraviolet irradiation (λ = 254 nm) differ in normal and BrdUrd-substituted DNA. In the present experiments either cells containing BrdUrd-substituted DNA (protocol A, see Material and Methods) or normal DNA (protocol B) were used for microirradiation in order to recognize possible differences of the types of DNA lesions involved. For both protocols the results shown in this paper are in good agreement with hypothesis (i).

MATERIAL AND METHODS

Cell Cultures and Treatment of Cells Before Microirradiation

Experiments were conducted using a derivative (650 A) of the M3-1 Chinese hamster cell line (30). This cell line has a modal chromosome number of 23 and a mean cell cycle transit time of approximately 11 hr (31). Stock cultures were maintained as described (21). For microirradiation experiments, cells were inoculated in plastic petri dishes (6 cm Ø). Prior to inoculation an "experimental field" (6 x 6 squares of 0.25 mm^2 each) was marked by scalpel cuts on the bottom of each dish. After inoculation, cells were grown for 1 cell cycle (11 hr) in minimum essential medium (MEM) plus 10% FCS plus 10 µg/ml BrdUrd (protocol A) or in MEM plus 10% FCS (protocol B) (32). For microirradiation, the medium was replaced by 2 ml HEPES-buffered serum free medium containing phenol red as a pH-indicator and the dishes were transferred into a special irradiation chamber (25).

Microirradiation Procedure

A continuous wave coherent UV-beam with wavelength 257 nm (24) was focused with a quartz microscope objective (Zeiss Ultrafluar 32x/0.40 Ph), which was simultaneously used for microirradiation and observation of the cells in phase contrast. For microirradiation of small nuclear areas or cytoplasmic areas at a distance of about 4 µm from the edge of the nucleus, the adjustment of the beam was made in such a way that the focal plane of the beam and the object plane of the microscope objective coincided. Thus, any cellular structure in focus of the microscope objective could be irradiated by the focal site of the beam. The beam's diameter was approximately 1 µm, as estimated from the smallest diameter of the fluorescent spot induced at the surface of a petri dish (33). The "effective" diameter of the irradiation field, however, was somewhat larger due to the divergence of the microbeam above and below its focal site and the effect of stray light produced when the beam passes a cellular structure. Indirect immunofluorescence microscopy using antibodies against UV-irradiated DNA revealed that microirradiated chromatin comprised an average of 4.5% of the total nuclear area. A frequency distribution curve of immunofluorescent nuclear areas showed a maximum at 2% (28). A frequency distribution curve of the individual

areas planimetered from 104 nuclei of living cells by use of a camera lucida showed nuclear areas between 80 and 180 μm^2 in 90% of the cells. The average nuclear area was 137 μm^2. By use of an adapting lens (24,25) the focal site of the beam could also be adjusted to different sites above the object plane. This resulted in "medium" or "large" circular microirradiation fields with a diameter of 7 or 12 µm, covering approximately 30 and 80%, respectively, of the total area of an average-sized cell nucleus. In nuclei of smaller size, the "large" microirradiation field resulted in irradiation of the whole nucleus. The actual diameter of the irradiation field was controlled by measuring the diameter of the fluorescent area produced at the surface of the petri dish with high intensity of the beam. Thus, a given UV incident dose could be distributed in different ways (irradiation modes "small," "medium," and "large") within the nucleus of a cell. Aiming was performed by means of a cross hair located in the image plane of the objective and adjusted to coincide with the center of the irradiation field. Horizontal movements of cells in irradiation chambers were controlled by using a special objective stage ("Gleittisch," Zeiss) which allows very fine adjustments. The irradiation times ranged from 1/125 sec to 1/15 sec; the UV power incident at the cell surface (25) was routinely 7.5×10^{-9} W. For low doses it was 4 or 2×10^{-9} W. Throughout the text, the incident energy E_{inc} = irradiation time x incident UV power is given. All cells (approximately 150) located in an "experimental field" were microirradiated at room temperature within approximately 30 min. Cells outside the "experimental field" served as controls.

Post-treatment of Microirradiated Cells

Protocol A. Cells grown in BrdUrd prior to microirradiation were post-incubated for 13 hr in BrdUrd-free medium (MEM plus 10% FCS). The incubation time was 2 hr longer than the normal cell cycle transit time to account for the microirradiation induced delay.

Protocol B. Cells containing unsubstituted DNA (no BrdUrd treatment prior to microirradiation) were post-incubated for 13 hr in MEM plus 10% FCS plus 10 µg/ml BrdUrd, followed by 11 hr incubation in BrdUrd-free medium All incubations were performed at 37°C in a humidified atomosphere with 5% CO_2. Necessary manipulations were carried out using a darkroom lamp with red filtered light. A red filter was also used for illumination of cells on the microscope stage. For the last 2 hr of post-incubation, colchicine (2 µg/ml final concentration) was added. Then "in situ" chromosome preparation was performed (34). To do this, the cells were treated with hypotonic solution (75 mM KCl) for 25 min. Thereafter, fixative (glacial acetic acid:methanol, 1:3) was added slowly. After 3 changes of fixative, the cells were air-dried. Differential chromatid staining was performed according to a modified fluorescence-plus-Giemsa method (35).

RESULTS

Microirradiation of the Nucleus

Figure 1 presents examples for the distribution of SCEs in metaphase spreads which were obtained in microirradiation experiments following protocol A. Figure 1a shows a typical control metaphase (no irradiation). Ten SCEs are visible in this cell. The chromosomes bearing these SCEs are not apparently clustered in the metaphase spread. If a small nuclear area (approx. 5% of the total nuclear area) was irradiated in the preceding interphase, metaphase spreads were occasionally observed with multiple SCEs over single chromosomal segments (Fig. 1b). Since such clusters of SCEs were not observed in the control cell population, we believe that they were induced by microirradiation. The number of UV-induced SCEs obtained after microirradiation of a small nuclear area, however, was small (Fig. 2a, 2b). Thus, UV-induced SCEs could often not be distinguished with sufficient confidence from "background" SCEs, when they were distributed over several chromosomal segments (see Discussion).

In other spreads, especially those exposed to higher doses, chromosome shattering was observed in a small part of the spread. This phenomenon is termed partial chromosome shattering (PCS) (Fig. 1c). In the intact chromosomes of these spreads, we did not note an apparent excess of SCEs above background levels. Evidence that the shattered chromatin in cells with PCS reflects the microirradiated chromatin has recently been provided using antibodies against UV-irradiated DNA (21). If a medium-sized nuclear area (approx. 30%) was microirradiated at 1 pole of the ellipsoid M3-1 nuclei, a high rate of SCEs was observed in a number of chromosomes which appeared generally clustered at 1 site of the metaphase spread (Fig. 1d). Following microirradiation of a large nuclear area (some 80% in average-sized nuclei and comprising the whole nuclear area in many smaller nuclei) most or even all chromosomes showed an increased level of SCEs (Fig. 1e).

Figure 2 shows frequency distribution curves for the number of SCEs per metaphase obtained after microirradiation of small and large nuclear areas, respectively, with 0.03 nJ each. An average of 12.2 SCEs was obtained after microirradiation of a small area, in contrast to 30.5 SCEs after microirradiation of a large one (controls 9.1 SCEs). In Fig. 3 the number of metaphase spreads from the same experiments is plotted as a function of the number Nc of chromosomes bearing SCEs in each spread. Nc was significantly larger after microirradiation of a large nuclear area ($\bar{N}c$ = 12.8) as compared to a small one ($\bar{N}c$ = 8.2; controls 7.3).

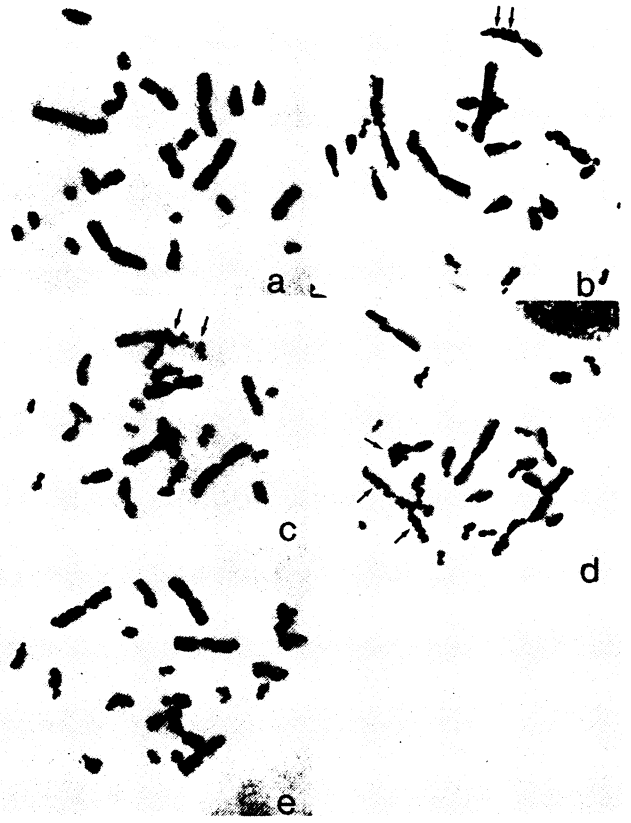

Fig. 1. Induction of SCEs at the first post-irradiation mitosis following laser-UV-microirradiation (λ = 257 nm) of interphase nuclei (protocol A). a) Control-metaphase (no irradiation) with 10 SCES. b) Metaphase following microirradiation of a small nuclear area ($\sim$ 5%). Arrows indicate a chromosome arm with multiple SCEs. Incident energy: E_{inc} = 0.015 nJ. c) Metaphase following microirradiation of a small nuclear area. Arrow indicates shattered chromosome material. E_{inc} = 0.03 nJ. d) Metaphase following microirradiation of a medium-sized area at the nuclear pole ($\sim$ 30 % of the total nuclear area). Arrows indicate peripherally situated chromosomes with a strongly increased number of SCEs. E_{inc} = 0.015 nJ. e) Microirradiation of approximately the whole nuclear area. Most chromosomes show multiple SCEs. E_{inc} = 0.03 nJ.

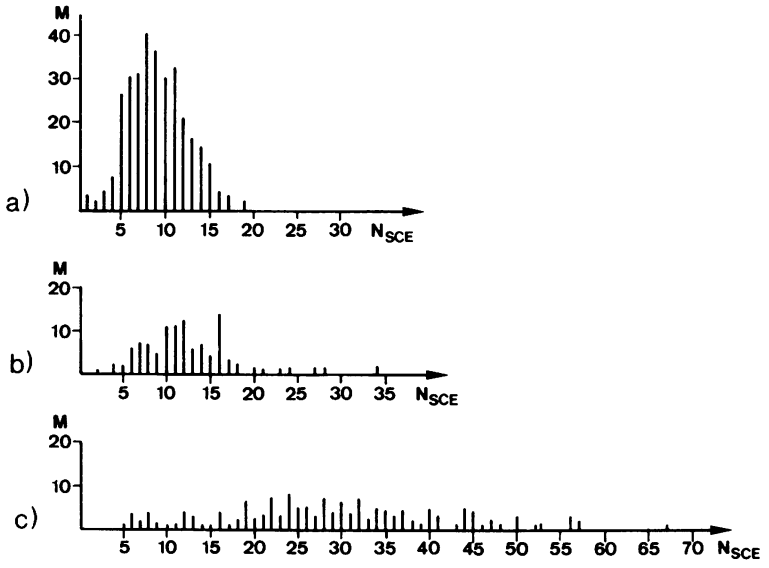

Fig. 2. Frequency distribution of the number of SCEs per metaphase. Chinese hamster nuclei with BrdUrd-substituted DNA were UV-microirradiated in interphase (incident energy: E_{inc} = 0.03 nJ. Chromosome preparations were obtained at the first post-irradiation mitosis (protocol A). Ordinate: number of metaphase spreads (M) with a given number of SCEs. Abscissa: number of SCEs per metaphase (N_{SCE}). a) Control (no irradiation). N_{SCE} = 9.1 ± 0.19; n = 312. b) Microirradiation of a small part (∿ 5%) of the nuclear area. $\bar{N}_{SCE}$ = 28.8 ± 0.49; n = 106. c) Microirradiation of a large part (∿ 90%) of the nuclear area. ($\bar{N}_{SCE}$: mean number of SCEs ± S.E.M.) In some cases included in the calculation of $\bar{N}_{SCE}$ the actual number of SCEs was probably slightly underestimated due to the quality of the spreads. However, elimination of these spreads from further calculations had a negligible impact on the values for $\bar{N}_{SCE}$ and we decided to evaluate the populations of metaphase spreads with SCD as complete as possible.

Table 1 summarizes the results of 1,198 metaphase spreads with sister chromatid differentiation (SCD) which were obtained following microirradiation of nuclei under different conditions. For both protocols A and B (see Material and Methods) and all incident UV energies (0.015 to 0.27 nJ), both Nc and the total number of SCEs obtained per metaphase spread with SCD were significantly higher when the UV energy was distributed over a large nuclear area instead of being concentrated to a small one. Although the incident UV doses used were considerably higher in experiments following protocol B, the increase of SCEs was less pronounced than in experiments following protocol A. These differences may partly reflect a lower

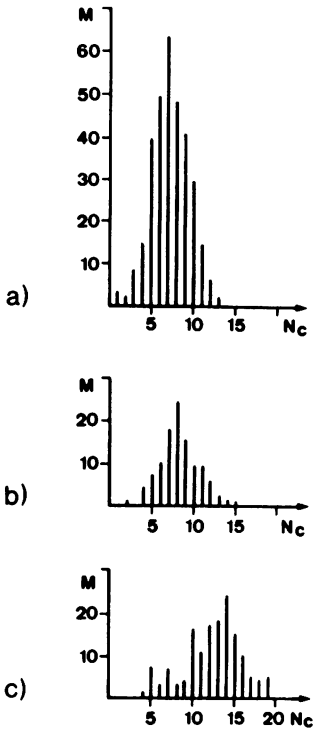

Fig. 3. Frequency distribution of the number of chromosomes per metaphase spread with SCEs. Chinese hamster nuclei with BrdUrd-substituted DNA were UV-microirradiated (incident energy: E_{inc} = 0.03 nJ). Chromosome preparations were obtained at the first post-irradiation mitosis (protocol A). Ordinate: number of metaphase spreads (M) with a given number of chromosomes bearing SCEs. Abscissa: number of chromosomes per metaphase spread bearing SCEs (N_C).
a) Control (no irradiation). $\bar{N}_C$ = 7.3 ± 0.12; n = 312.
b) Irradiation field "small." $\bar{N}_C$ = 8.2 ± 0.22; n = 106.
c) Irradiation field "large." $\bar{N}_C$ = 12.3 ± 0.28; n = 154.
($\bar{N}_C$ = mean number of chromosomes bearing SCEs ± S.E.M.).

sensitivity of nonsubstituted DNA (protocol B) as compared to DNA substituted with BrdUrd at the time of microirradiation (protocol A) (36). PCS was only detected after microirradiation of small and medium nuclear areas following protocol A, but not in cells with SCD obtained by protocol B. With regard to PCS, one has to take into consideration that the first postirradiation mitosis was evaluated in case of protocol A, while in case of protocol B cells with SCD had proceeded through 2 additional cycles after microirradiation. Cells with shattered chromosomes do not survive but are still able to form micronuclei (our unpublished observations).

Tab. 1. SCE formation following microirradiation of small, medium, and large areas Chinese hamster nuclei.

BrdUrd/dT labeling protocol	incident UV energy (nJ)	irradiation field	n	n_{SCD}	$\frac{n_{SCD}}{n} \times 100$	N_C (mean ± S.E.M.)	N_{SCE} (mean ± S.E.M.)	N_{MN}
A	control	--	--	312	--	7.3 ± 0.12	9.1 ± 0.19	3.6
	0.015	small	799	86	10.8	9.8 ± 0.29	16.7 ± 0.71	7.9
		medium	928	80	8.6	14.8 ± 0.62	38.0 ± 2.88	10.0
	0.03	small	1.936	106	5.5	8.2 ± 0.22	12.2 ± 0.49	7.6
		large	1.910	161	8.4	12.3 ± 0.28	28.8 ± 1.02	4.0
	0.06	small	1.126	29	2.6	9.8 ± 0.65	13.3 ± 1.13	12.6
		large	1.169	67	5.7	16.4 ± 0.38	54 ± 1.69	6.6
B	control	--	--	267	--	7.0 ± 0.14	9.4 ± 0.27	3.5
	0.13	small	3.011	98	3.3	7.2 ± 0.24	10.8 ± 0.44	15.8
		large	1.255	177	14.1	9.3 ± 0.20	15.8 ± 0.48	7.6
	0.27	small	3.256	154	4.7	7.0 ± 0.18	10.1 ± 0.31	24.3
		large	1.519	241	25.8	9.6 ± 0.18	16.8 ± 0.49	9.8

n : number of microirradiated cells

n_{SCD} : number of metaphases with SCD

N_C : number of chromosomes with SCEs per metaphase

N_{SCE} : total number of SCEs per metaphase

N_{MN} : percentage of micronucleated cells

Interestingly, the distribution of a given UV incident dose into a small or large nuclear area resulted in a striking difference both with regard to the mitotic yield, i.e., the number of metaphases with SCD divided by the number of microirradiated cells, and the percentage of cells with micronuclei. After microrirradiation of small nuclear areas the mitotic yield was generally lower and the percentage of micronucleated cells higher. This result suggests that cellular survival may not only depend on the total incident UV dose applied to the nucleus (25) but also on its distribution within the nucleus. Further support for this suggestion was obtained by the observation that clonal survival of microirradiated cells was improved after microirradiation of larger nuclear areas as compared to smaller ones (our unpublished data). Notably, microirradiation of a small part of the nucleus with the lowest UV-incident dose (Tab. 1, protocol A, 0.015 nJ) resulted in a significantly higher yield of SCEs per metaphase spread at the first postirradiation mitosis than microirradiation with higher doses (0.03 and 0.06 nJ). This is likely due to a more pronounced chromatin damage in cells microirradiated at higher doses which possibly interfered with the formation of SCEs or led to an underestimation of SCEs in microirradiated chromosomal segments which appeared shattered at metaphase.

Microirradiation of the Cytoplasm

In microirradiation experiments of nuclear targets the microbeam also passed the layer of cytoplasm above and below the nucleus. To exclude SCE induction by toxic and/or DNA-damaging effects of diffusible photoproducts ("radiotoxins") produced outside the nucleus (37) a small part of the cytoplasm of M3-1 cells was microirradiated at a distance of about 4 µm to the edge of the nucleus. The results are given in Tab. 2. No significant differences were

Tab. 2. SCE formation following microirradiation of a small part of the cytoplasm beside the nucleus.

BrdUrd/dT labeling protocol	incident UV energy (nJ)	n_{SCD}	N_C	N_{SCE}
A	0.27	45	7.2 ± 0.31	9.6 ± 0.49
B	0.27	27	7.0 ± 0.46	9.2 ± 0.60

for unirradiated controls see Table 1

n_{SCD} : number of metaphases with SCD

N_C : number of chromosomes with SCEs

N_{SCE} : total number of SCEs per metaphase

obtained between control cells and cells microirradiated in the cytoplasm. In experiments following protocol A the UV-incident dose applied to the cytoplasm was considerably higher (approx. 3-30 times) as compared to the doses used for microirradiation of nuclei. This compensates for the differences in the UV-absorbance of nucleoplasm (approx. 50%) and cytoplasm (approx. 30% of the UV-incident energy) (38). It is concluded that the significantly increased SCE levels obtained after microirradiation of small parts of nuclei at the first postirradiation mitosis (protocol A) were exclusively due to direct effects on nuclear targets.

DISCUSSION

In the present investigation we have tried to discriminate by laser-UV-microbeam experiments between 2 alternative hypotheses concerning the induction of SCEs by ultraviolet light (see Introduction):

(i) SCEs are formed exclusively at or near the sites of DNA photolesions.

(ii) The number of SCEs depends on the total number of DNA photolesions, but the sites of such lesions and the sites of SCEs are not correlated.

Both the number and the distribution of SCEs were found to be dramatically influenced by the distribution of the UV-incident energy within the nucleus (Figs. 1-3, Tab. 1). Our interpretation of these data is based on evidence that chromosomes in the interphase nucleus of Chinese hamster cells occupy distinct territories or domains (26-28,33,39,40). Recently, Hens et al. (28) in our laboratory, microirradiated small areas (some 5% of the total area) using the same cell line (M3-1) as in the present experiments. The cells were followed from interphase to metaphase and the microirradiated chromatin was visualized by indirect immunofluorescence microscopy using antibodies specific for UV-irradiated DNA. The frequency distribution of immunofluorescent chromosomes showed a maximum of 2 labeled chromosomes per metaphase spread and an arithmetic mean of 3.1 chromosomes, i.e., parts of these chromosomes were situated in the microirradiated nuclear segment. In light of these findings we suggest that multiple SCEs found over single chromosomes after microirradiation of a small nuclear area (Fig. 1b) indicate microirradiated chromosomes. In cases, however, where the few microbeam-induced SCEs were distributed over several microirradiated chromosomal segments it became difficult or impossible to distinguish microirradiated chromosomes from nonirradiated ones bearing "background" SCEs. Our conclusion that the induction of SCEs by ultraviolet light is restricted to microirradiated chromatin is substantiated by our

finding that even in case of partial chromosome shattering (PCS) induced by microirradiation of a small nuclear area, the surrounding intact chromosomes showed apparently normal SCE levels (Fig. 1c). After microirradiation of a medium-sized nuclear area (some 30%), multiple SCEs were noted over a number of chromosomes apparently rather close to each other in the metaphase spread, while the remaining chromosomes again showed SCE levels in the range of controls (Fig. 1d). Finally, after microirradiation of a large nuclear area, covering the major part or even the whole nucleus, many metaphase spreads could be observed with high SCE levels over the majority or even all chromosomes (Fig. 1e).

For both protocols A and B our data are clearly consistent with hypothesis (i) but not with hypothesis (ii). This finding indicates that the different types of DNA lesions produced by UV-irradiation of normal and BrdUrd-substituted DNA induce SCEs by a mechanism acting directly within the microirradiated chromatin. Although we cannot exclude the induction of a small percentage of SCEs in replicon clusters remote from the UV-irradiated ones, we can now safely assume that such indirect effects do not play a major role under the present experimental conditions.

Recently, Graves and Kellow have assayed sister chromatid exchange frequencies in heterokaryons between irradiated and unirradiated mouse and Chinese hamster cells (42). One cell line was UV-irradiated, then fused to unirradiated BrdUrd-labeled cells of the other line. SCEs in the unirradiated chromosome complement were scored in heterokaryons. In these experiments a dose dependent increase of SCEs in the unirradiated chromosome complement of the heterokaryons could be demonstrated. The authors have suggested from their data that an indirect pathway may account for 25% of SCEs induced in UV-irradiated cells. In addition, Graves and Kellow have discussed the possibility that a factor which induces SCEs in the nonirradiated chromosomes might be produced in UV-irradiated cytoplasm. In the present experiments microirradiation of the cytoplasm beside the nucleus did not induce the formation of SCEs (Tab. 2). However, it is important to note that only a small amount of cytoplasm was microirradiated. Thus we cannot exclude the possibility that irradiation of a major part or even the whole cytoplasm might lead to an increased extent of indirect SCE induction. In contrast to Graves and Kellow (42), Gaitil and his coworkers did not observe an increase of SCE frequency in nonirradiated BrdUrd-labeled chromosomes in hybrid cells resulting from the fusion of unirradiated and UV-irradiated V79 Chinese hamster cells.

Within the range of energy densities used in the present experiments it is likely that the total number of DNA photolesions was approximately the same after microirradiation of small and large nuclear areas, respectively (23). One might have expected that only

the distribution of SCEs over the chromosome complement, but not their total number, should be influenced by these 2 modes of microirradiation. In part, the much lower total yields of SCEs observed in the first case (Fig. 2) may be due to an underestimation of possible SCEs in shattered chromosomes (Fig. 1c). However, this explanation does not appear sufficient, since the differences in the yields of SCEs observed after both modes of microirradiation were still present in metaphase spreads with apparently intact chromosomes. Estimates of the maximum density of SCEs over single chromosomes, indicate slightly higher values at best in the case of a small irradiation field (data not shown). This contrasts to a maximum energy density which should be at least 50-fold higher in a small microirradiation field as compared to a large one. We have especially looked for chromosome segments in which differential staining of sister chromatids might have become blurred due to a high number of local SCEs resulting from high local energy densities, but we were not able to detect such examples. Thus, the observed frequencies of SCEs over single microirradiated chromosomes do not apparently reflect the differences in the local energy densities obtained by the different diameters of the irradiation field. Assuming an irradiation area of 2% of the mean nuclear area, i.e., the immunofluorescent area most frequently observed when the focal site of the beam was placed within the nucleus (Ref. 28; see Material & Methods) an energy density in the range of 100 J/m^2 can be estimated in the microirradiated nuclear part. This rough estimate suggests that the energy densities obtained by microirradiation of a small nuclear area were generally in a range where considerable saturation of SCE numbers was observed in case of whole cell irradiation with conventional UV sources (λ = 254 nm) (35,41). In contrast, the much lower energy densities achieved by exposing a large nuclear area were likely in a range still below saturation. Besides saturation, a high number of DNA photolesions in microirradiated chromosomes may interfere with the formation of SCEs by inducing other pathways by which a cell can cope with UV-induced chromatin damage and which result in other endpoints observable at mitosis, such as chromosome shattering.

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