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Laser Microirradiation of Chinese Hamster Cells at Wavelength 365 nm: Effects of Psoralen and Caffeine

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Cells of a V79 subline of the Chinese hamster were microirradiated at wavelength 365 nm in the presence of the psoralen derivative, trioxsalen. Microirradiation was accomplished by a pulsed argon laser microbeam either in anaphase or in interphase 3 hr after mitosis. Inhibition of clonal growth and formation of micronuclei at the first postirradiation mitosis were observed after microirradiation of anaphase chromosomes and of small parts of the interphase nucleus. Microirradiation of the cytoplasm beside the interphase nucleus or between the sets of chromosomes moving apart from each other in anaphase did not produce these effects. Anaphase experiments showed that only the daughter cell which received microirradiated chromatin exhibited an abnormal growth pattern. Most interestingly, shattering of the whole chromosome complement could be induced by microirradiation of small parts of the interphase nucleus and post-treatment with caffeine. Since microirradiation of chromatin in the absence of psoralen was not effective, we consider formation of psoralen photoadducts to nucleic acids in microirradiated chromatin to be the specific cause of the effects. We suggest that DNA photolesions in chromosome segments present in the microirradiated part of the nucleus can induce shattering of all the chromosomes in the microirradiated nucleus. Several possibilities are discussed to explain this unexpected finding.

INTRODUCTION

Models which have been proposed to explain the synergistic effect of uv irradiation and caffeine in producing chromosome damage and cell death are based on the finding that caffeine interferes with daughter-strand repair of uv-damaged templates (*1–4*). Formation of chromosome aberrations is expected to start within uv-damaged DNA strands at or near the sites of DNA photolesions (*5*).

Recently, microirradiation experiments performed with a uv laser microbeam at wavelength 257 nm have been reported (*6–8*).[2] The hypothesis has been derived

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[2] T. Cremer, C. Cremer, C. Zorn, and J. Zimmer, The use of a UV-laser microbeam to test a model for the induction of chromosome aberrations by ultraviolet light. In *Abstracts, Helsinki Chromosome Conference, August 29–31, 1977*, p. 168.

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from these experiments that replication of chromosomes which bear a sufficiently high number of DNA photolesions may lead to damage of unirradiated chromosomes present in the same nucleus. This hypothesis is based on two findings:

1. Recent evidence supports the idea that chromosomes in the interphase nucleus of Chinese hamster cells are arranged in rather compact territories (*9–11*). This means that the microbeam hits only a few chromosome segments when we microirradiate a small part of the interphase nucleus.

2. Microirradiation at wavelengths 257 (*6–8*)[2] and 261 nm (unpublished data) of a randomly selected small part of the chromatin in interphase nuclei of Chinese hamster cells and post-treatment with caffeine often result in mitotic cells where all the chromosomes are shattered (generalized chromosome shattering, GCS). Microirradiation of the cytoplasm does not produce this effect. Mitotic cells with GCS show a severe fragmentation or even pulverization of the chromosomes. Fragmented and pulverized chromosomes may be present in the same cell. GCS has been introduced as a descriptive term to avoid any prejudice with regard to the interpretation of this effect (*12*).

In support of the above hypothesis, it must be shown that it is actually the effect of DNA photolesions produced in microirradiated chromosomes which influences the morphological integrity of both irradiated and unirradiated chromosomes present in the same nucleus. As an approach to answering this question we have performed microirradiation experiments with Chinese hamster cells at wavelength 365 nm in the presence of psoralen (PUVA microirradiation). By this treatment, monofunctional and bifunctional photoadducts are formed depending on whether a single psoralen molecule binds to one or both strands of RNA and DNA, respectively (*13–15*). The specificity of the photoadducts for the observed effects can be demonstrated by showing that neither UVA irradiation nor psoralen alone is effective. Under appropriate conditions microirradiation permits limitation of the formation of photoadducts to nucleic acids in a selected small part of a cell and thus allows for the identification of the subcellular localization of target molecules (*16*).[3] Here we report effects of PUVA microirradiation and caffeine post-treatment on clonal growth, chromosome shattering, and formation of micronuclei in Chinese hamster cells.

MATERIALS AND METHODS

Cell material and culture conditions. Cells of a V79 Chinese hamster subline, V79-122D1 (*17*), were grown in Eagle's minimum essential medium supplemented with 10% fetal calf serum (FCS) (GIBCO) in a humidified atmosphere with 5% CO_2 at 37°C. The average generation time was 13 hr. For irradiation experiments, cells in mitosis were harvested by shake-off from Falcon T-75 flasks and seeded into Rose chambers at low cell densities. Conditioned medium obtained from exponentially growing V79 cultures 2 days after subcultivation and supplemented with an additional 5% of fresh FCS was used to further cultivate these cells.

Psoralen and caffeine. Psoralen used in these experiments was 4,5',8-

[3] S. P. Peterson, Ph.D. thesis, University of California, Irvine, 1979.

trimethylpsoralen (trioxsalen) at a concentration of 0.6 μg/ml (*15*). Trioxsalen was applied to cells in Rose chambers about 30 min before irradiation. After irradiation, medium was replaced by conditioned medium free from psoralen. Addition of 1 and 2 m*M* caffeine was employed as described under Results.

Microbeam. Microirradiation was performed using a Hughes argon laser microbeam at wavelength 365 nm as previously described (*16*).[3] The laser output at 365 nm was 0.6 W with a pulse duration of 25 μsec. The focal diameter of the microbeam was between 0.5 and 1.0 μm.

Microirradiation of cells. Due to the aperture angle of the focusing objective and the thickness of the cell, the cellular area hit by the microbeam was larger than the focus of the beam. Despite this fact, 10% or less of the total nuclear area of V79 cells could be microirradiated selectively. Normally, an area of a cell selected for microirradiation was exposed to three pulses of laser light after the light had been passed through an 0.8% transmission filter.

Microirradiation of cells in interphase was performed approximately 3 hr after mitosis when cells were in G1 and early S phase, respectively. In other experiments, mitotic cells were selected between 12 and 14 hr after seeding and microirradiated at a time when anaphase movement of the chromosomes became visible in phase contrast. In interphase, either nucleoplasm or cytoplasm beside the nucleus was selected for microirradiation. Nucleoli were excluded from microirradiation. In anaphase, chromatin or cytoplasm between the moving sets of chromosomes was microirradiated. Localization of microirradiated cells was marked with a circle on the outer surface of the glass slide. The fate of these cells was followed individually by phase contrast observations with a Zeiss photomicroscope II or III. An air curtain incubator served to maintain the Rose chambers at 37°C on the microscope stage.

Whole-cell irradiation with UVA. If not stated otherwise Rose chambers were placed approximately 30 cm from a General Electric fluorescent uv tube No. 630T8 for different periods of time (5–30 min). The glass coverslips on which cells were grown served as a filter against far uv so that the cells were exposed to the UVA fraction of the tube. In some experiments a germicidal lamp (Sterisol 5143, Original Hanau) was used with appropriate filtering to eliminate UVB/UVC. Pre- and post-treatment of the cultures were the same as described for microirradiated cells.

Clonal growth assay. Clonal growth was assumed when a cell was able to produce a colony of more than 50 cells. In most cases growth of colonies was followed until they could be seen by the naked eye. In some cases both daughters of a single mitotic cell were microirradiated in the same way. Growth of both cells was individually followed thereafter to establish whether one or both cells contributed to colony formation. In other cases one daughter cell was destroyed by a number of laser pulses at high intensity, while the other daughter was used for the experiment. Data from both types of experiment were pooled.

Chromosome preparation. Chromosome preparations were performed when cells entered the first postirradiation mitosis. When early prophase became visible, 0.05 μg/ml colcemid were added for an additional 2–3 hr. Preparation was performed *in situ*, i.e., without removal of cells from the substrate as described elsewhere (*11*).

TABLE I

Microirradiation of Chinese Hamster Cells in Interphase (G1/Early S Phase): Inhibition of Clonal Growth

Irradiation site	*Trioxsalen (0.6 μg/ml)*	*Caffeine post-treatment (1 mM)*	*Number of cells irradiated*	*Clonal growth*
No irradiation	−	−	44	42 (95%)
	−	+	44	44 (100%)
	+	−	26	26 (100%)
	+	+	50	49 (98%)
Nucleus	−	−	20	19 (95%)
	−	+	27	27 (100%)
	+	+	30	7 (23%)[a]
Cytoplasm	+	+	20	20 (100%)

Note. Experiments were performed using three pulses of laser light with 0.8% transmission filter. Presence of trioxsalen during irradiation and post-treatment with caffeine, respectively, is indicated by +.

[a] Indicates significant inhibition of clonal growth.

Determination of DNA content. Cells were stained by the standard Feulgen-Schiff reaction (*18*). The 442-nm wavelength of a helium–cadmium laser was employed to stimulate fluorescence in Feulgen-stained metaphase plates. A nanometrics Nanospec 10 microspectrofluorometer set to read fluorescence at 620 nm was used to record quantitatively the fluorescence produced by metaphase plates with intact and shattered chromosomes, respectively (*19*).

RESULTS

Microirradiation of Cells in Interphase

Clonal growth. Table I shows the effect on clonal growth when a randomly selected small part of either the nucleoplasm or cytoplasm of V79 cells was microirradiated in G1/early S phase. In the presence of psoralen (PUVA microirradiation), the fraction of cells which were able to form a clone decreased after microirradiation of the nucleus, while microirradiation of the cytoplasm did not affect cellular growth. Microirradiation of the nucleus in the absence of psoralen did not inhibit clonal growth of the cells even when the dose was increased by two orders of magnitude above the range routinely used in the present series of experiments. After PUVA microirradiation of the nucleus at higher doses than those used for the Table I data, the fraction of cells with clonal growth was reduced to zero both with and without caffeine post-treatment. At the concentrations used in the present experiments, psoralen and caffeine did not reduce the plating efficiency of unirradiated cells.

Induction of generalized chromosome shattering in microirradiation and whole-cell irradiation experiments. In situ chromosome preparations performed at the first postirradiation mitosis of cells after microirradiation of a small part of the

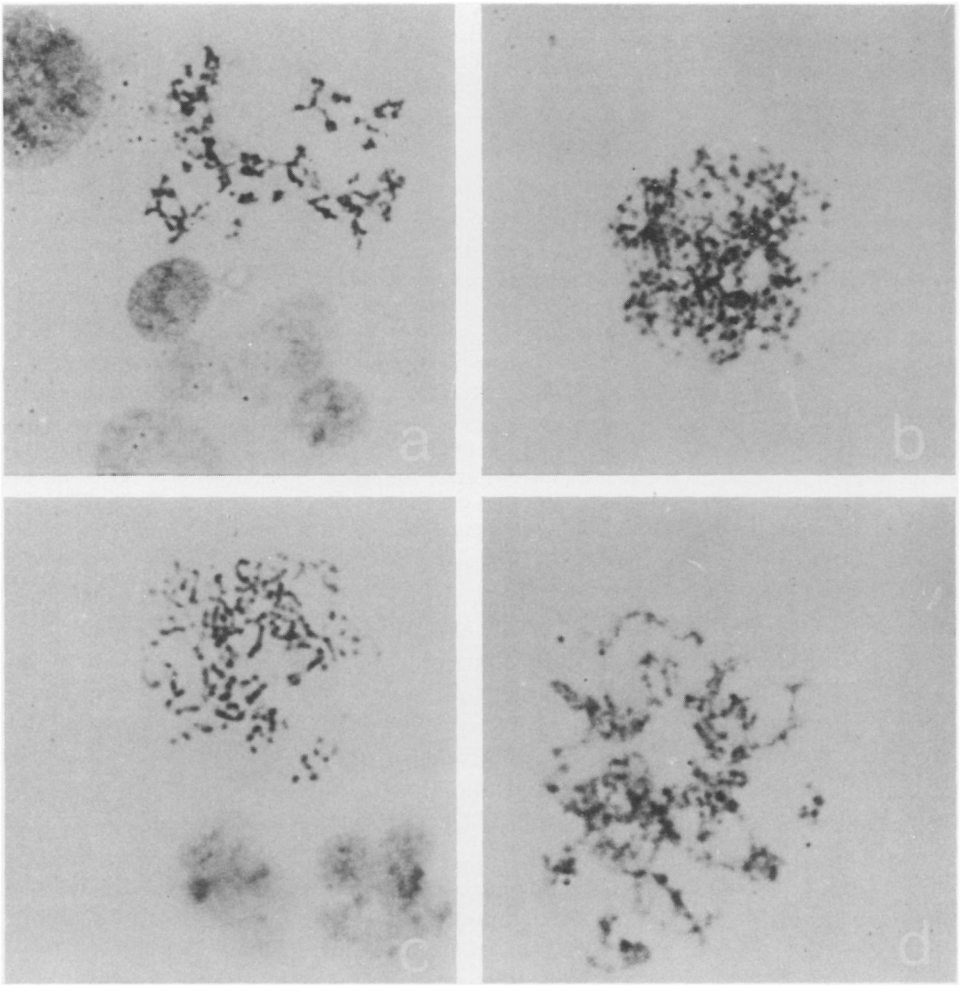

FIG. 1. Metaphase plates with shattered chromosomes obtained after microirradiation of the nucleus of a V79 cell in G1 and caffeine post-treatment (2 m*M*). Three pulses of laser light (0.8% transmission filter) were applied in the presence of trioxsalen (0.6 μg/ml); stained with Feulgen (a, b) or methylene blue (c, d).

nucleus in G1/early S phase revealed that *all* the chromosomes of a cell frequently became shattered (generalized chromosome shattering, GCS) (Fig. 1) when microirradiation was performed in the presence of psoralen and with caffeine post-treatment (20/51 cases) (Table II). PUVA microirradiation of the cytoplasm and caffeine post-treatment, however, did not induce chromosome shattering.

Considerable overlapping of chromosomes was observed in many metaphase plates in this series of microirradiation experiments. Therefore an exact account of single aberrations was not feasible. Notably, in numerous cells which escaped GCS after PUVA microirradiation of the nucleus and caffeine post-treatment (31/51 cases), the chromosome complement appeared intact.

TABLE II

Microirradiation of Chinese Hamster Cells in Interphase (G_1/Early S Phase): Induction of Chromosome Shattering in First Postirradiation Mitosis

Irradiation site	*Trioxsalen (0.6 μg/ml)*	*Caffeine post-treatment (2 mM)*	*Number of cells irradiated*	*Total number of metaphase plates*	*Number of metaphase plates with shattered chromosomes*
Nucleus	–	–	43	27	0
	+	–	45	21	0
	+	+	106	51	20
Cytoplasm	+	+	27	18	0

Note. Cells were microirradiated at a randomly selected site in the nucleoplasm or in the cytoplasm a few μm away from the nuclear edge. Further proliferation of each cell was individually followed by phase contrast observation, and preparation of metaphase plates was performed *in situ* at the first postirradiation mitosis. Due to technical problems, the number of metaphase plates obtained does not equal the total number of microirradiated cells which proceeded to mitosis.

Whole-cell irradiation of V79 cultures with UVA in the presence of psoralen (PUVA irradiation) presented further evidence that GCS is produced by a synergistic effect of caffeine on cells containing psoralen photoadducts in nucleic acids. In cells which were treated with psoralen or UVA alone, caffeine (1–2 m*M*) did not induce GCS. PUVA irradiation of synchronized cultures in G1/early S phase (i.e., 3 hr after these cultures were established by mitotic harvest) produced a high percentage of GCS at the first postirradiation mitosis (up to 80% at the highest dose investigated) when the cells were post-treated with 2 m*M* caffeine, while the percentage of GCS was low (up to 5%) when the caffeine post-treatment was omitted. Notably, cells which escaped GCS often showed only a few aberrations or even no aberrations at all. PUVA irradiation plus caffeine post-treatment (2 m*M*) of unsynchronized cultures showed that shattered chromosome complements were delayed; i.e., GCS was not observed in mitotic samples 6 hr after irradiation. After 12 and 18 hr, respectively, 18 and 19% of the mitotic cells showed GCS after a shorter radiation exposure (6 min, Sterisol 5143). These percentages increased to 68 and 52% after a longer radiation exposure (12 min). The percentages of apparently intact metaphase plates were 49 and 58%, respectively, at the lower dose and decreased to 13 and 27% at the higher dose. Percentages were calculated from evaluation of 100 mitotic cells in each experiment.

A pronounced mitotic delay (several hours) was observed in PUVA-treated cells, which was prevented by caffeine post-treatment. In the absence of caffeine, cells which did not divide often became unusually large ("giant cells") after both PUVA irradiation and PUVA microirradiation of the nucleus. Giant-cell formation was not observed after PUVA microirradiation of the cytoplasm and was prevented by caffeine post-treatment after both PUVA irradiation and PUVA microirradiation of the nucleus. These findings will be described in detail elsewhere (S. P. Peterson *et al.*, in preparation).

As can be seen from Table III no differences between the amount of DNA in

TABLE III

DNA Content of Mitotic Cells with Intact or Shattered Chromosomes (GCS) Obtained after PUVA Irradiation in G1/Early S Phase and Caffeine Post-treatment

	Number of metaphase plates	*Average fluorescence of Feulgen stain recorded by the microfluorometer*	*Range*
Chromosomes shattered	51	11.3 ± 0.9	9.0–13.1
Chromosomes intact	43	11.1 ± 0.8	8.5–12.6

mitotic cells with shattered chromosomes and the amount of DNA in normal metaphase plates were detected. While slight differences in DNA content may have escaped notice, these findings suggest that cells with shattered chromosomes had replicated the bulk of DNA following PUVA irradiation in G1/early S phase and caffeine post-treatment.

Formation of micronuclei. PUVA microirradiation of the nucleus often resulted in the formation of micronuclei [for definition see (*20*)]. The percentage of micronucleated cells increased from 13 to 60% after post-treatment with 2 m*M* caffeine (Table IV). Phase contrast observations of microirradiated cells as well as cells from cultures which had received PUVA irradiation revealed that formation of micronuclei was the result of an abnormal first postirradiation mitosis. In some cases mitotic cells which became micronucleated did not undergo cytokinesis, while in other cases micronucleated daughter cells were produced. Evidence that cells with GCS are still able to produce micronuclei will be presented elsewhere (T. Cremer *et al.*, in preparation).

Microirradiation of Cells in Mitosis

Microirradiation of mitotic cells at a time when the nuclear envelope was absent allowed further testing of whether or not the effects described above could be attributed directly to microirradiation of chromosomes present in the microir-

TABLE IV

Microirradiation of Chinese Hamster Cells in Interphase (G_1/Early S Phase): Formation of Micronuclei in First Postirradiation Mitosis

Irradiation site	*Trioxsalen (0.6 μg/ml)*	*Caffeine post-treatment (2 mM)*	*Number of cells irradiated*	*Number of cells with formation of micronuclei*
Nucleus	–	–	32	0
	+	–	61	8 (13%)
	+	+	173	104 (60%)
Cytoplasm	+	+	50	0

TABLE V

Microirradiation of Chinese Hamster Cells in Anaphase: Formation of Micronuclei in First Postirradiation Mitosis

Area irradiated	*Number of cells irradiated*	*Number of cells micronucleated*	*Percentage of total*
Interzone region of cytoplasm	25	1	4
Chromatid	25	12	45

Note. All above experiments were performed using three pulses of laser light in presence of trioxsalen (0.6 μg/ml) with 0.8% transmission filter; cells were post-treated with 2 m*M* caffeine in conditioned medium.

radiated part of the nucleus or depended on other effects of irradiation (e.g., alteration of the nuclear envelope or stray light).

In anaphase, when the chromatids separated and moved apart, either some chromatin or an interzone region between the moving sets of chromosomes was microirradiated in the presence of psoralen. Cells were post-treated with 2 m*M* caffeine. No immediate effect of microirradiation was noted and both daughter cells showed normal phase contrast morphology. The further development of cells, however, clearly differed depending upon whether or not a cell had received microirradiated chromatin (Table V). In the first case, the next mitosis was often abnormal and resulted in the formation of micronuclei in 12 out of 25 experiments, while in the latter case, normal cell divisions were observed (Fig. 2a, b).

Microirradiation of anaphase cells in the interzone region resulted in normal divisions of both daughter cells. Micronucleation was observed in 1 out of 25 experiments. Most probably, some chromatin was accidentally microirradiated in this case.

Microirradiation of chromatin in metaphase has been performed in only a few cases. At this stage of the cell cycle, both chromatids of the microirradiated chromosomes are similarly affected. Figure 3 shows the result of such an experiment. Two daughter cells, each containing a nucleus of normal size, were formed. The subsequent mitosis, however, was grossly abnormal in both cells and resulted in the formation of micronuclei. The possibility must be considered that some other metaphase chromosomes situated close to the microirradiated one were also affected by the microbeam. The anaphase experiments, however, clearly indicate that the action of the microbeam is restricted to the microirradiated part of the cell.

DISCUSSION

Effects of PUVA irradiation on chromosome structure and clonal growth have been reported previously (*21*, *22*). Our results indicate that inhibition of clonal growth, chromosomal damage, and formation of micronuclei at the first postirradiation mitosis of Chinese hamster cells can be induced by psoralen photoadducts in microirradiated chromatin, while binding of psoralen to nucleic acids in a small part of the cytoplasm produces little or no effect. Previously, it had

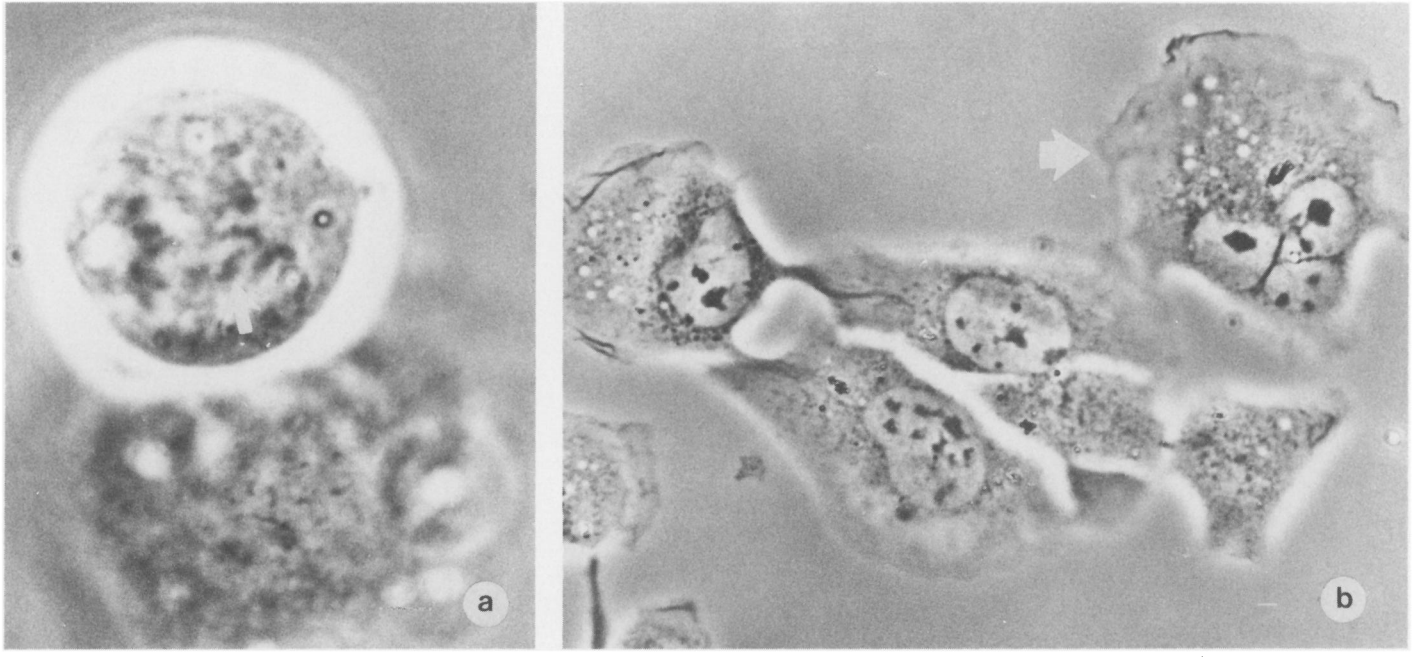

FIG. 2. (a) Phase contrast picture of a living V79 cell in anaphase. Some chromatin (arrow) of the cell was microirradiated in the presence of trioxsalen (0.6 μg/ml) with three pulses of laser light using 0.8% transmission filter. This picture was taken immediately after irradiation. No alteration was observed at the irradiation site and the cell finished mitosis in an apparently normal manner. An adjacent cell was destroyed by a number of laser pulses at high intensity (no transmission filter). Trioxsalen-containing medium was replaced by conditioned medium with caffeine (2 m*M*) after the daughter cells had been formed. Bar = 10 μm. (b) Thirty-five hours later, the daughter cell with the unirradiated chromosome complement had produced five cells, indicating normal clonal growth. The other daughter cell which had received microirradiated chromatin is indicated by the arrow. As the result of an abnormal first postirradiation mitosis, the nucleus of this cell fragmented into three micronuclei. Cytokinesis was not observed in this case. In some other cases, however, formation of daughter cells containing micronuclei was found. Bar = 10 μm.

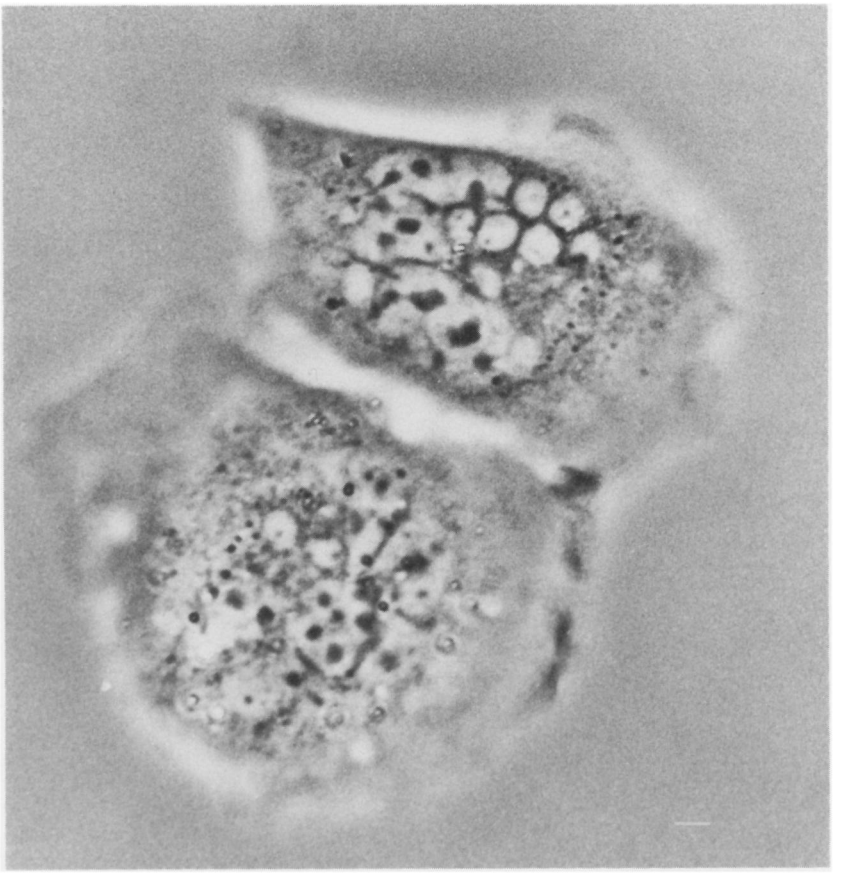

FIG. 3. Phase contrast picture shows two daughter cells 24 hr after microirradiation of a chromosome in the metaphase plate of a living V79 cell. Both chromatids of the microirradiated metaphase chromosome(s) were hit by the microbeam; otherwise, conditions of microirradiation and caffeine post-treatment were the same as in the experiment shown in Fig. 2. No adverse effect of microirradiation on the subsequent stages of mitosis was observed. The subsequent mitosis, however, was abnormal in both daughter cells. Formation of numerous micronuclei may suggest that the delayed effect of microirradiation was not restricted to microirradiated chromatin. Bar = 10 μm.

been demonstrated that PUVA microirradiation of the centriole region, but not of other cytoplasmic regions, affects mitosis in cells of *Potorous tridactylis* (*16*). In the present experiments, the chance of microirradiation of the centriole region was small since the site of microirradiation in both the nucleoplasm and cytoplasm was chosen at random. The specific role of psoralen photoadducts in nucleic acids, most probably chromosomal DNA, is suggested by the fact that neither psoralen alone nor microirradiation at wavelength 365 nm alone produced these effects. The percentage of cells in which formation of micronuclei was observed after PUVA microirradiation of the nucleus increased strongly when the cells were post-treated with caffeine. Most interestingly, all the chromosomes of a cell frequently became shattered (generalized chromosome shattering, GCS) by the synergistic effect of

PUVA microirradiation of a small area of the interphase nucleus and caffeine post-treatment.

DNA lesions produced far from the irradiation site by the action of stray light can be excluded as a sufficient explanation of GCS, since GCS was not observed after PUVA microirradiation of cytoplasm beside the interphase nucleus or mitotic chromosomes. Microirradiation of chromatin in anaphase provides a particularly sensitive test for possible effects of stray light. Cellular growth of the daughter cell which did not incorporate the microirradiated chromatin remained unaffected although the chromosomes which moved to this daughter cell were in part only a few micrometers away from the irradiation site. It may be noted, however, that it is necessary to establish a suitable dose range at which biological effects of stray light can be neglected. When the irradiation energy was raised by two orders of magnitude above the range chosen for the present experiments, inhibition of cellular growth of both daughters of mitotic cells could be observed after PUVA microirradiation of either anaphase chromosomes or interzone regions. It is likely that this finding can be simply interpreted as a result of stray light.

In previous experiments performed at wavelength 257 nm the possibility that some diffusible irradiation products were formed in the microirradiated part of the nucleus has been taken into consideration. These products might have affected the chromatin of the whole nucleus and induced GCS (*6*). Present knowledge concerning the molecular effects of PUVA treatment renders such an explanation rather unlikely in the case of PUVA microirradiation (*13–15*). The following hypothesis is established as a consequence of present as well as previous microirradiation experiments (*6–8*).[2] Photochemically induced DNA lesions (e.g., psoralen photoadducts, thymidine dimers) produced in microirradiated chromosome domains of the interphase nucleus (*9–11*) may under certain conditions induce shattering of unirradiated chromosomes situated in domains remote from the irradiation site. Different lines of reasoning (see (a)–(e) below) can be followed to interpret our findings in light of such a hypothesis, and the following discussion is not meant to be exhaustive. A major difficulty of interpretation stems from the fact that caffeine produces pleiotropic effects at both the cellular and molecular levels (*1*, *23*). In particular, the possible relationship between the effects of caffeine on replication of damaged DNA and its ability to increase the frequency of uv-induced chromosomal aberrations is far from clear (*1–3*,*23*). Evidence for a remote action of DNA lesions makes the picture even more complicated.

(a) Genetic information important for the morphological integrity of *all* mitotic chromosomes may be blocked by transcription-terminating photolesions in microirradiated chromatin (*24*). Caffeine might prevent such genes from transcription by interfering with daughter-strand repair. If this interpretation were correct, it would follow from these and other experiments [(*6*); C. Cremer *et al.*, in preparation] that many chromosome domains in the cell nucleus should contain such hypothetical genes. Alternatively we may consider the idea that GCS is induced when the number of DNA photolesions in microirradiated chromatin becomes sufficiently large, even if this chromatin does not contain any genes essential for the structural integrity of chromosomes. In principle, either induction (synthesis?, activation?) of some chromosome breakage or unwinding factors ((b)

and (c)), or depletion of factors involved in chromosome replication ((d) and (e)) may play a role.

(b) Increased DNA degradation after uv irradiation has been reported for mammalian cell systems and several mutants in bacteria (*25*, *26*) and may take place in the microirradiated nucleus as well. Loss of control of such a degradation process may occur in the presence of caffeine and result in shattering of both irradiated and unirradiated chromosomes (*6*). Shattered chromosomes then should reflect high numbers of true breaks. On the other hand, the morphology of shattered chromosomes often closely resembles SPCC chromosomes (*27*). Shattered chromosomes may therefore indicate that chromosome condensation was forced before DNA replication was completed. The measurement of DNA content (Table III) provides an argument against this idea. However, the sensitivity of the Feulgen method employed was not sufficient to detect the missing replication of a small amount of DNA. Furthermore, the argument that more extended chromatin might result in enhanced Feulgen staining and obscure differences in the DNA content of normal mitotic cells and cells with GCS cannot be ruled out at the moment. Further studies are planned to clarify this point. We strongly consider the possibility that biosynthesis of both microirradiated and unirradiated chromatin was altered in a way which prevented its normal condensation and thus resulted in GCS.

(c) It is known that conformational changes of chromatin and nuclear DNA are induced by DNA lesions (*28*, *29*). The possibility that DNA lesions in a small part of the nucleus may induce conformational changes in both microirradiated and unirradiated chromatin may be considered, although we do not know of any evidence in support of such an idea. The sensitivity of unwound chromatin against effects of caffeine, which is known to produce GCS even in unirradiated cells at high concentrations [(*1*); unpublished observations], might be considerably enhanced in this state in both the microirradiated and unirradiated parts of the nucleus.

(d) Caffeine has been reported to have effects on DNA precursor pools (*30*). We have previously shown that the percentage of cells with GCS obtained after whole-cell irradiation ($\lambda = 254$ nm) and caffeine post-treatment of V79 cells can be significantly reduced by adding the four deoxyribonucleosides to the medium (*12*). Drainage of DNA precursor pools might interfere with DNA replication both in microirradiated and unirradiated chromatin. However, it is questionable whether precursor pools were appreciably affected by 2 m*M* caffeine (*23*). Instead the antagonistic effect of nucleosides on the induction of GCS may be due to enhanced DNA repair synthesis (*12*, *31*). If so, the number of DNA photolesions still present at the time of replication of damaged chromatin, i.e., at the time when caffeine is thought to exert its synergistic effect on GCS induction (*1*), becomes smaller in nucleoside-treated cells than in untreated ones.

(e) A final consideration concerns the possibility that certain catalytic factors *F* may be involved in replication of both damaged and undamaged chromatin (*7*).[2] Such factors have been found in *Escherichia coli* (*32*–*35*). *F* may be a catalytic factor acting both at DNA replication forks and at sites of daughter-strand repair. The mechanisms of daughter-strand repair are still controversial (*36*), but for the purpose of the present discussion it can be defined as the process by which synthesis of daughter strands can be completed in the presence of DNA lesions in the parental strands (*4*). Essentially, DNA lesions should give rise to factor binding

sites N_{uv} only at a certain stage during the course of daughter-strand repair. Additional binding sites N_0 should be offered by all DNA replication forks. F is taken from a pool when a new binding site is created and released into the pool after having completed its particular function. Drainage of the pool may then be the critical event in the induction of GCS. Drainage takes place when the number of factor binding sites N surpasses a threshold N_T regardless of the distribution of N within the nucleus (*8*). Since N_{uv} depends on the incident uv energy E_{uv} applied to the cell nucleus, we propose the following sequence of events: A threshold energy E_T defines the maximum energy which a given cell can tolerate under certain experimental conditions and still escape chromosome shattering. More precisely, E_T correlates with the highest tolerable load of DNA lesions, i.e., a critical number of psoralen photoadducts in the case of PUVA treatment. In case $E_{uv} > E_T$ it follows $N > N_T$ and chromosome shattering occurs. DNA lesions which can be repaired before the replication fork approaches the lesion site do not contribute to drainage of the pool (see (d)). According to this factor depletion model caffeine should increase the number of binding sites N so that $N > N_T$ occurs even after moderate uv doses. Present knowledge concerning the effects of caffeine on DNA replication fits well into such a concept. Caffeine prevents the uv-induced inhibition of DNA replication by increasing the number of DNA growing points, i.e., more origins are used in both irradiated and unirradiated DNA (*23*). At the cellular level caffeine decreases the mitotic delay after PUVA treatment in G1/early S phase and other DNA-damaging agents as well (*37*). Thus in the presence of caffeine the number of binding sites formed per time during replication should moderately increase in the unirradiated part of the nucleus and strongly in the PUVA-microirradiated part. Lehmann and Kirk-Bell (*38*) have interpreted the results of pulse-chase experiments with [^{3}H]thymidine to indicate a gap-filling process in newly synthesized DNA opposite DNA lesions in the parental strand. Caffeine was found to specifically inhibit this process (*38*). Although alternative interpretations seem possible (*36*) this result suggests that caffeine may inhibit the termination of binding sites N_{uv}. Both mechanisms of caffeine action could lead to drainage of the pool. As a result, misreplication, which has been assumed to be essentially involved in the production of chromosomal aberrations by agents with delayed effects (*39*), may take place in *all* chromosomes of a microirradiated cell. Both the molecular nature of this misreplication and the chain of events which lead from misreplication to GCS remain obscure. When viewing possible candidates for F (e.g., polymerases, ligases, etc.) in light of different models of daughter-strand repair, it should be kept in mind that drainage of the pool should not interfere with replication of the bulk of DNA but with a more subtle process. Variation of the pool size in individual cells would provide an easy explanation of why an apparently identical treatment of cells at a certain stage of the cell cycle may induce GCS in some cells but not in others (*40*). A full discussion of this factor depletion model will be presented elsewhere in light of further experimental data (C. Cremer *et al.*, in preparation).

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