BEHAVIOUR OF THE MITOTIC APPARATUS AFTER ULTRAVIOLET MICROIRRADIATION OF THE SPINDLE POLE*

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The results of the effect of u.v. microirradiation of the centrosome (mitotic spindle pole) on cell division in the metaphase and anaphase are discussed. It is shown that the response of the cell essentially depends on the stage of mitosis at which the pole is microirradiated. Irradiation of the pole in the metaphase blocks the cell in mitosis for several hours, whereas irradiation in the anaphase does not impede exit from mitosis. Irradiation in the metaphase of one of the poles leads to an immediate shift of all the chromosomes to the non-irradiated pole. Then the spindle is completely destroyed. Irradiation of one the poles in the anaphase only slows the movement of the chromosomes to the irradiated pole without, in practice, affecting the movement of the chromosomes to the opposite pole. Ultrastructural investigation showed that the irradiated centrosome loses the link with the kinetochore bundles of the chromosomes and in the metaphase draws nearer to the chromosomes while in the anaphase it shifts to the periphery of the cell. Control micro-irradiation of other regions of the cell does not lead to the effects described. © 1996 Elsevier Science Ltd. All rights reserved.

In animal cells the mitotic spindle is organized by two centrosomes (spindle poles), although their concrete role in the regulation of the work of the mitotic apparatus has still not been elucidated [1]. This is largely explained by the technical complexity of inactivation or selective damage to the centrosome [2]. Microirradiation is the most adequate instrument for this task. The use for microirradiation of the light in the visible range (400-700 nm) has the shortcoming that because of the low absorption of living cells, it is necessary either to use very high radiation densities or to sensitize the cells by introducing dyes [2]. Staining may produce changes unrelated to microirradiation in cell physiology, making it difficult to interpret the results. Therefore, we opted for microirradiation with far u.v. light, which is well absorbed by non-sensitized cells [4-5].

Experiments with exposure of the spindle pole of division to u.v. microirradiation were, in the main, carried out in the 1960s [6-9], with equivocal results. Takeda and Isutsu [6] were the first to investigate the effect of microirradiation of the centrosome on the behaviour of the cells. They established the formation of numerous vacuoles on irradiation of the pole in the metaphase and

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disturbance of the radial position of the mitochondria after irradiation at later stages. In addition, irradiation of a portion of the spindle may produce a rapidly reversible shift in some of the chromosomes from the irradiated pole. Nakanishi and Kato [7] irradiated a portion of the spindle containing the pole in the telophase. After such irradiation the chromosomes shifted to the non-irradiated pole passing the equator, with disappearance of the groove of division, staining of the cell and blocking of cytotomy. In [9] Zirkle states that the destruction of the spindle of division in the mitotic cells of the pericardial mesothelium of the triton occurs on irradiation of different regions of the cell and does not depend on the presence of the centrosome in the irradiated area. Unlike other authors, Sakharov and Voronkova [8] noted no changes in the course of mitosis after u.v. microirradiation of the spindle pole of division in swine kidney embryo cells. Recently, new findings appeared on the effect of focused u.v. irradiation. A number of investigations showed destruction by focused u.v. light of the microtubules in vitro and in living cells [10-14], the action spectrum in both cases being practically identical [12].

Earlier, we devised an apparatus for u.v. microirradiation enabling us to damage selectively a portion of the cell with a diameter of not more than 2 µm [15]. It turned out that microirradiation of the centrosome leads to certain anomalies in the course of mitosis and in the case of passage of the cell to the interphase, the centrosome is not the centre of organization of the microtubules [16]. The aim of the present work was to make a more detailed study of the behaviour of the centrosome and the chromosomes in the realization of the functions of the spindle in its two states — in the early metaphase and during divergence of the centrosomes in the anaphase.

MATERIALS AND METHODS

As test objects, we used PEK tissue culture cells. The cells were grown on round quartz cover slips which were then mounted into the Dvofak-Schtottler modified chamber for vital observations. For culturing, we used medium 199 with 10% embryonic calf serum and an antibiotic (gentamycin).

For microirradiation a special unit was used [15]. Irradiation was through an interference light filter (λ_{max} = 280 nm, Δλ_{1/2} = 10 nm) using an Ultrafluar 100/1.25 quartz objective with glycerol immersion (Opton, Germany). The diameter of the light (u.v.) beam in the focal plane of the objective was 1.6 (µm. The power density of irradiation was about 4 X 10^{-2} erg/(µm/s). The vital observations of the cells were made in phase contrast (Plan Neofluar 40/0.9 objective, glycerol immersion, Opton, Germany). The cells were photographed with the MFN-12 microphotocap on Mikrat-izopan film (Tasma, Russia) or observed by a television system.

For statistical treatment of the results, 20 cells were irradiated at the pole of each stage of mitosis, and in them we determined the mean speeds of movement of the chromosomes for 10 min after irradiation of the pole. For electron microscopic fixation of the cells, the chamber was dismantled, the glass washed in physiological phosphate buffer (PBS, pH 7.2) at 37°C, then the cells fixed in 2.5% glutaraldehyde in PBS. They were then prefixed with 1% OsO₄, contrasted in a solution of uranyl acetate, dehydrated and embedded in EPON-812 mixture by the standard technique.

Serial ultrafine sections were prepared on the LKB-3 ultramicrotome (Sweden), mounted on to diaphragms, covered with a formvar support, stained with lead citrate after Reynolds, inspected and photographed on HU-11B and HU-12 electron microscopes (Japan) at an accelerating voltage of 75 kV.
RESULTS

Ultraviolet microirradiation of the centrosome (time of irradiation 5 s) in PEK cells was carried out in the early metaphase (5 min after formation of the metaphase plate) and in a higher dose (15 s) in the early anaphase (1 min after the start of the anaphasic movement of the chromosomes). As well as the centrosome (spindle pole), as controls at the same doses we irradiated the chromosomes, portions of the cytoplasm outside the spindle of division and within it (Fig. 1).

Microirradiation of the pole at the start of the metaphase shifted the chromosomes towards the non-irradiated pole (Fig. 2). The speed of the shift of the marginal portions of the metaphase plate far exceeded that of its central part (0.73 ± 0.14 μm/min as against 0.09 ± 0.03 μm/min), as a result of which the plate bent around the non-irradiated pole and all the chromosomes proved to be equidistant from it. The kinetochore bundles of the intact semi-spindle after irradiation of the pole shortened and the centrosome drew nearer to the chromosomes. The distance between the pole and chromosomes during 15 min after irradiation was about 1.5 μm. The metaphase plate disintegrated 15—20 min after irradiation.

Electron microscopy showed that 1 min after irradiation of the pole, the microtubules around it were arranged in unordered form. Three to five minutes after irradiation of the pole the kinetochore bundles of the microtubules did not approach it. Within 20 min the non-irradiated centrosome was in a ring of chromosomes but that irradiated outside was also close to the chromosomes. Control u.v. microirradiation of the chromosomes (seven cells) and the cytoplasm (17 cells) in higher doses (15 s) did not lead to disintegration of the spindle and did not influence the normal completion of mitosis.

Microirradiation of the centrosome in the early anaphase at once slowed the movement of the chromosomes towards the irradiated pole (Fig. 3). Their speed was 0.50 ± 0.11 μm/min as compared with 1.19 ± 0.11 μm/min in the sister chromosomes, the movement of which to the non-irradiated pole was not disturbed (the speed of movement of the chromosomes in the control cells in the anaphase 2 min after the start of divergence was 1.30 ± 0.06 μm/min). Cytotomy in the cells with one irradiated pole came 10—15 min later than in the control. A constriction formed strictly equidistant from the two groups of chromosomes, so that the cell receiving the irradiated

Fig. 1. Regions of microirradiation (schematized): 1, spindle pole (centrosome); 2, chromosomes; 3, portion of spindle between pole and chromosomes; 4, portion of cytoplasm outside the spindle.
Fig. 2. Tracing (a) and graph of the movement (b) of the chromosomes in the metaphase. (a) The contours of the metaphase plate are given before irradiation and 1, 7 and 10 min after it. The irradiated pole is on the right. Abscissa, distance (in microns) from the centre of masses of the metaphase plate at the moment of radiation, (b) 1, Movement of the edges of the plate; 2, movement of the centre of the plate. $L$, distance covered by the chromosomes in relation to the position of the metaphase plate.

The centrosome after division was somewhat larger. Irradiation in the anaphase of the other regions of the cytoplasm did not lead to the effects described above.

Electron microscopy showed that in the anaphase 1 min after irradiation of the centrosome (time of irradiation 15 s), the bundles of microtubules fell short of it by 1.5—2 µm. Then (3 min after irradiation) the irradiated pole "floated away" from the chromosomes, being situated twice as far from them as the opposite pole. There were practically no microtubules around it. The kinetochore bundles from the irradiated pole gradually shortening persisted up to the start of cytotomy.

DISCUSSION OF RESULTS

To solve the problem of the role of the centrosome in meta- and anaphase mitosis, it is desirable to compare our results on microirradiation of the centrosome with similar experiments with irradiation of the spindle of division [11, 17—19]. Our previous work [20] showed that u.v. microirradiation of the centrosome in the anaphase is capable of leading to its functional inactivation in the interphase — disturbance of its ability to polymerize the microtubules. In the present work we investigated the direct effects of irradiation, starting from the notion that the centrosome is known to be damaged by microirradiation. The radiation doses in our experiments (0.2-0.6 erg s/µm²) were close to those used in work with sectioning of the microtubules [11, 17, 19].

After microirradiation in the metaphase, the response of the cell to irradiation of bundles of microtubules and irradiation of the centrosome fundamentally differs. Irradiation of bundles of microtubules in the metaphase leads to their sectioning, the pole shifting to the equator [11, 17, 19]. Then the link between the pole and its semi-spindle is restored through growth of the microtubules into the zone of irradiation from the centrosome [19] or from the kinetochores [11,
Fig. 3. Tracing of the movement (a) and graph (b) of the chromosomes in the anaphase. (a) The contours of the anaphase chromosomes are given before irradiation and 7 and 10 min after it. Irradiated pole is on the right. (For symbols see Fig. 2a.) (b) 1, Movement of the control chromosomes; 2, movement of the chromosomes to the non-irradiated (opposite) pole; 3, movement of the chromosomes to the irradiated pole. $L$, distance covered by the chromosomes in relation to the position of the metaphase plate.

Microirradiation of the centrosome, unlike irradiation of the bundles of microtubules, leads to irreversible after effects — dismantling of the semi-spindle from the irradiated pole and simultaneous shift of the chromosomes to the opposite one.

Thus, the centrosomes in the metaphase are capable of restoring the damaged spindle by initiating polymerization of new microtubules, and inactivation of one of them by microirradiation leads to degradation of the whole spindle. The shift of the chromosomes indicates the presence of a balance of forces applied to the opposed kinetochores from the semi-spindles. Apparently, the sharp weakening of tension induced by irradiation of the pole in the metaphase serves as a signal for the start of work and dismantling of the opposite semi-spindle.

In the anaphase, microirradiation slows down more than twofold the movement of the chromosomes to the irradiated pole. Nevertheless, the anaphase is completed. Similar results were obtained on microsurgical removal of the spindle pole in cricket spermatoocytes [21] and on sectioning the kinetochore bundles by u.v. microirradiation [19]. Unfortunately, it is not possible for us to compare the quantitative characteristics of the movement of the chromosomes after irradiation of the pole and sectioning of the kinetochore bundles, since in the last case the authors
simply confine themselves to the statement that the chromosomes continued their movement (along changed trajectories) [19].

Immediately after irradiation of the pole (centrosome) in the metaphase, it begins to come closer to the chromosomes. On the other hand, in the anaphase the pole after irradiation moves away from the chromosomes. Since in both cases the irradiated centrosome lost the link with the microtubules, it must be assumed that its displacement in the cell is regulated not only by the microtubules but also by other systems.

Thus, in the metaphase the centrosome is a necessary element of the spindle and damage to it has irreversible consequences. Starting from the anaphase, the role of the centrosome changes. It remains an essential element in the following interphase but no longer influences the process of divergence of the chromosomes and the exit of the cell from mitosis.

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