レーサー・マイクロビームを照射したラト・カンガルー細胞(PT-KI) における仁形成の抑制

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SHORT COMMUNICATION

INHIBITION OF NUCLEOLAR FORMATION IN PT-KL RAT-KANGAROO CELLS BY LASER MICROBEAM IRRADIATION[1)]

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Inhibition of nucleolar formation in salamander lung cells, after laser microbeam irradiation of chromosomes, was reported in a previous paper (Berns *et al.* 1970). Microbeam irradiation of the secondary constrictions in anaphase chromosomes caused a reduction in nucleolar number in reconstructed daughter nuclei. This indicated that the argon laser microbeam system is an efficient tool to analyze genetic function of specific sites of large urodele chromosomes, and Berns *et al.* (1971) have demonstrated that it can be applied to produce non-specific lesions in small mammalian chromosomes. The mammalian cell would be the system of choice to study persistence of a genetic defect in the progeny of an irradiated cell, since its generation time is short in comparison to the 5- or 6-day cell cycle of the salamander cell. Therefore, we have selected the female rat-kangaroo (*Potorous tridactylis*) cell line Pt-Kl to extend our studies, because they demonstrate: (1) a small chromosome number (modal number is 11); (2) a conspicuous secondary constriction which is generally observed on both X-chromosomes; (3) a clear morphology and a fairly consistent number of nucleoli (usually 2 nucleoli, but sometimes 1 large nucleolus which was apparently derived from 2 fused nucleoli, were observed in 1928 out of 2,000 nuclei in regular diploid size-cells, in a 5-day culture); (4) well-spreading epithelial type cells. These characteristics make it possible to identify the secondary constrictions of X-chromosomes easily, even in phasecontrast images of living cells, especially at anaphase. Hsu *et al.* (1967) clearly demonstrated that the nucleolus organizer (NO) is located at these secondary constrictions of Pt-Kl cells as a special structure.

Cells were cultured in Rose chambers with the medium composed of Eagle's minimum essential medium (MEM) with nonessential amino acids, sodium pyruvate, and Earle's balanced salt solution (BSS) with reduced bicarbonate (0.85 gm/liter) plus 10% fetal calf serum. The experiment was carried out under the air-curtain incubator system, which kept the chambers at 37°±0.5°C. Cells were photosensitized to the laser light by 3 to

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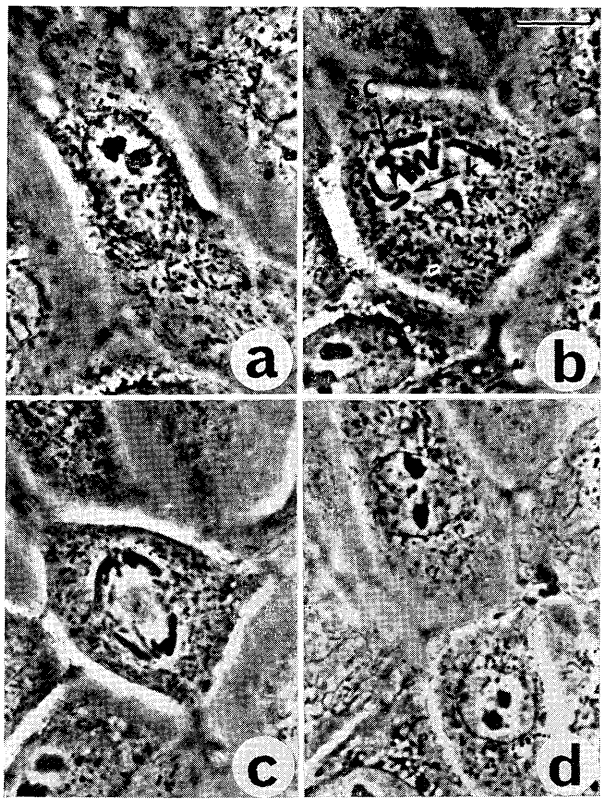

Fig. 1a–d. Illustration of the consistency in the nucleolar number of Pt-Kl rat-kangaroo cells in the mitotic process. Scale line represents 10 μ.

(a) Early prophase. Note 2 clear nucleoli. (b) Early metaphase (36 minutes after Fig. 1a). One X-chromosome is clearly shown, with arrows indicating the secondary constriction (SC) and kinetochore (k); (c) Late anaphase (52 minutes); (d) Interphase (111 minutes). Both daughter nuclei formed 2 nucleoli.

5 minutes incubation in 0.1 to 0.001 μg/ml acridine orange (AO) in Gey's BSS before irradiation. Cells were washed once or twice with normal BSS and cultured in the medium described above. The same argon laser microbeam system was used, as described in previous papers (Berns *et al.* 1970; Ohnuki *et al.* 1972). It consisted of an argon laser (Hughes Aircraft Model 3040 H) combined with a Zeiss Photo-microscope. Irradiation of secondary constrictions was carried out during anaphase. Generally, the whole region of each secondary constriction (the constriction itself and adjoining regions) was irradiated 5 to 7 times. The region of the secondary constriction in only 1 daughter chromosome was irradiated, and the corresponding unirradiated daughter SAT-chromosome served as the control. A chromosome region not containing a secondary constriction was irradiated in the control side of the daughter chromosome group with the same dosage as applied to the secondary constriction. Nucleolar formation was subsequently observed, and cells were recorded by phase-contrast photomicroscopy or time-lapse

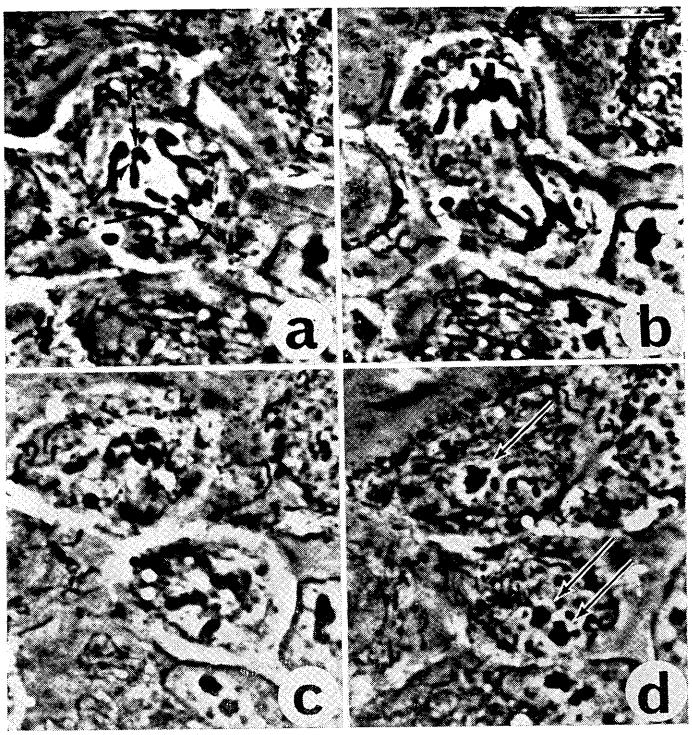

Fig. 2a-d. Laser micro-irradiation of the region of the secondaryconstriction of 1 X-chromosome in anaphase and subsequent nucleolar formation in daughter nuclei. Scale line represents 10μ.
(a) Anaphase (1 minute before irradiation). Note a pair of sister chromatids of the X-chromosome with clear secondary constrictions (SC) and kinetochores (k). Upper one was irradiated. (b) Early telophase (1 minute after irradiation); (c) Late telophase (7 minutes after irradiation); (d) Interphase (48 minutes). Only 1 nucleolus was formed in the irradiated nucleus, while 2 nucleoli were produced in the control nucleus.

cinematography for about 3 days.

Fig. 1 illustrates the consistency of the nucleolar number during mitotic process. Two large nucleoli were clearly visible in early prophase (Fig. 1a). About 30 minutes after this stage, nucleoli disappeared completely and chromosome condensation rapidly progressed. Almost simultaneously, breakdown of nuclear membrane took place and the cell went into early metaphase stage. The secondary constriction of the X-chromosomes was often seen very clearly in this stage (Fig. 1b). After the formation of metaphase plate, chromosomes stayed in the equatorial plate for about 10 to 15 minutes until the onset of anaphase movement. They reached each pole within 4 to 5 minutes. During this period, daughter chromosomes showed symmetrical arrangements and movement, which made it easy to identify individual chromosomes. When the cell was well spread and the chromosomes were not heavily overlapped, the secondary constriction of X-chromosomes usually were visible. Unfortunately, the secondary constriction was not clearly shown in Fig. 1c, because of the overlapping of chromosomes. One hour after

the disappearance of nucleoli in late prophase, nucleoli began to form and finally 2 nucleoli were completely formed in each daughter nucleus 30 minutes later (Fig. 1d).

Ten cells were irradiated at the region of secondary constriction of 1 daughter X-chromosome in anaphase, and subsequent nucleolar formation was observed in their daughter nuclei. Fig. 2 illustrates one example. At anaphase a pair of daughter X-chromosomes were clearly shown, with arrows indicating the secondary constrictions (before irradiation) (Fig. 2a). In this case, the region of secondary constriction in the upper daughter chromosome set was irradiated. Fig. 2b shows the cell after irradiation. A paling generally occurred in the region of the irradiated secondary constrictions; however, it was usually not so obvious as in the salamander chromosomes, because Pt-Kl chromosomes were small and moved much faster to the pole and overlapped each other quickly (Fig. 2b). The completion of the cytokinesis and the beginning of the formation of nuclear membrane are shown in Fig. 2c. About 30 minutes after irradiation, nucleoli began to form in each daughter nucleus. Finally, only 1 nucleolus was formed in the irradiation side (top), while 2 nucleoli were clearly formed in the control nucleus (bottom) (Fig. 2d). The results indicated that the irradiation of the region of the secondary constriction of an anaphase X-chromosome inhibited the formation of 1 nucleolus in the daughter nucleus. Ten replicate experiments offered the same result. A similar tendency of compensatory regulation mechanism of nucleoli was also observed in this experiment, but not as clearly as in salamander cells (Berns *et al.* 1970).

It has been observed, repeatedly (Berns *et al.* 1971; Ohnuki *et al.* 1972), that the cell cycle can be followed after random microbeam irradiation of chromosomes (not in the NO region). This gave evidence to prove that this treatment did not produce any serious secondary effects, with respect to the concentration of AO (less than 0.1 μg/ml) and the energy density of microbeam applied. The production of a paled spot in a non-specific region of chromosomes did not cause serious change in the mitotic process.

These results suggest a system which could establish whether the chromosomal lesion is replicated in subsequent generations. If the descendants of the irradiated cell maintains the reduced nucleolar number, it can be assumed that the localized damage is not repairable. It would then be technically feasible to develop cloned populations of cells with pre-selected damage sites, in order to identify the loci of a variety of genetic activities.

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