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Establishment of nucleolar deficient sublines of PtK2 (Potorous tridactylis) by ultraviolet laser microirradiation

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exponential kinetics. A similar conclusion was drawn earlier by Pfeifer [9] and Schworer et al. [13] using liver cells as experimental system.

The half-life of autophagic vacuoles estimated from the semilogarithmic plot was 5.8 min and the first-order rate constant 0.120 min^{-1} . Since 0.25% of the cytoplasmic content of control cells was present in the autophagic vacuole compartment (see table 1), a $0.120 \times 60 \times 0.25 \approx 1.8\%$ fraction of their cytoplasm was removed by autophagy each hour under steady-state conditions.

This value is higher than those observed in the intact liver [9] and in cultured cells [4, 6], but is close to that observed by Schworer et al. [13] in perfused liver during accelerated proteolysis. This similarity may not be accidental, since intracellular degradation was probably also enhanced in the seminal vesicle cells by fasting and addition of estrone acetate.

The estimated half-life of autophagic vacuoles in the seminal vesicle cells reported here was close to that observed in the liver [9, 13]. This may indicate that the velocities with which the elements of cytoplasm engulfed by an autophagic vacuole undergo destruction are similar in the different cells. If this is so, the autophagic process may primarily be regulated through the sequestration step.

References

1. Amenta, J S & Brocher, S C, *Life sci* 28 (1981) 1195.
2. Ballard, J F, *Essays biochem* 13 (1977) 1.
3. Bigelow, S, Hough, R & Rechsteiner, M, *Cell* 25 (1981) 83.
4. Dean, R T, *Protein turnover and lysosome function* (ed H L Segal & D J Doyle) p. 29. Academic Press, New York (1978).
5. Dice, J F, Bouret, L & Miao, P, *J cell biol* 91 (1981) 214 a.
6. Hendil, K B, *Exp cell res* 135 (1981) 157.
7. Kovács, A L & Seglen, P O, *Biochim biophys acta* 676 (1981) 213.
8. Marzella, L & Glaumann, H, *Lab invest* 42 (1980) 18.
9. Pfeifer, U, *J cell biol* 78 (1978) 152.
10. — *Acta biol med germ* 40 (1981) 1619.
11. Réz, G & Kovács, J, *Virchows arch B zellpathol* 12 (1973) 123.
12. Rumpelt, H J & Weisbach, T, *Am j pathol* 91 (1978) 49.
13. Schworer, C M, Schiffer, K A & Mortimore, G E, *J biol chem* 256 (1981) 7652.
14. Seglen P O, Gordon, P B, Grinde, B, Solheim, A, Kovács, A L & Poli, A, *Acta biol med germ* 40 (1981) 1587.
15. Weibel, E R, *Int rev cytol* 26 (1969) 235.
16. Woodside, K H, *Biochim biophys acta* 421 (1976) 70.

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Establishment of Nucleolar Deficient Sublines of PtK2 (*Potorous tridactylis*) by Ultraviolet Laser Microirradiation

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Summary. One of the two nucleoli of tetraploid PtK2 WA cells in early prophase was irradiated with an ultraviolet (UV) laser microbeam. The daughter cells that maintained the

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nucleolar deficiency were isolated and cloned. Five nucleolar deficient sublines of PtK2 WA were established, thus providing an experimental system to study the ribosomal gene-nucleolar organizer complex.

Since 1969, a series of studies involving laser microbeam irradiation of mitotic chromosomes in tissue culture cells was undertaken [1–4]. These early experiments demonstrated that it was possible to irradiate selectively the nucleolar organizer region of the chromosome and thereby produce post-mitotic cells with deficiencies in various numbers of nucleoli on the basis of how many nucleolar regions were irradiated. In 1972, it was reported that a newly developed tunable wavelength laser microbeam could provide wavelengths at a wide variety of energy densities from the ultraviolet (UV) (240 nm) through the entire visible portion of the spectrum [5]. Meanwhile, it was possible to minimize the damage and produce desired chromosome lesions without using any vital stain [6, 7, 8] when either UV or visible wavelengths were used.

The tetraploid subline PtK2 WA derived from the male rat kangaroo, *Potorous tridactylis*, possesses several advantageous features which facilitate laser irradiation. They have two nucleoli per cell and remain relatively flat during mitosis, thus making the microirradiation of the nucleolar organizer region easy to do [9]. Using this cell line, a clone of cells maintaining the nucleolar deficiency was isolated from a single cell in which one nucleolar genetic region was irradiated [11].

In the present study a series of experiments was conducted by focusing the UV laser microbeam onto one of the nucleolar organizer regions in cells of the PtK2 WA subline. The purpose of this study was to establish sublines of PtK2 cells with selectively induced nucleolar deficiencies and to examine the stability of these deficiencies as well as the growth and survival of the cell populations.

Material and Methods

Cell culture. The cells used in this study are from a tetraploid subline designated PtK2 WA that was derived from near diploid male rat kangaroo PtK2. This line has maintained the tetraploid condition for over 5 years. Most of the PtK2 WA cells have two nucleoli per cell. The cells are grown as monolayer cultures in a modified Eagle's medium containing 0.85 NaHCO₃ per liter, supplemented with 10% fetal calf serum (FCS) in T25 flasks prior to seeding into Rose multi-purpose culture chambers for irradiation. One glass coverslip and other quartz coverslip (thickness of 0.32 mm) manufactured by Esco Company (Oakridge, N.J.) were used for the chamber windows. Cells growing on the quartz coverglass were irradiated with the UV laser beam.

Laser irradiation. The microbeam system utilizes a Quantronix no. 116R pulsed neodymium-YAG laser with an output energy of 5 kW at the second harmonic wavelength of 532 nm. The green laser beam (532 nm) was frequency shifted to 266 nm by passage through a fixed frequency ADP second harmonic generator, Chromatix model no. 1050. The final laser output at 266 nm was 500–1000 W with a pulse duration of 80 nsec. Experiments were conducted using 266 nm laser light.

The UV beam of laser light was next deflected into a Zeiss photomicroscope by an especially designed, highly UV-reflective dichroic filter. The cell image was then projected onto a high light sensitivity television camera (RCA no. TC 1000). A television monitor with a cross-hair depicted the point of laser focus. The target area of the cell was then moved under the cross-hair on the monitor screen, and the laser was triggered by remote control.

Isolation and cloning of cells. Following irradiation, the cells were followed until they completed mitosis. After the two post-mitotic daughter cells, each with one nucleolus, were produced, the Rose

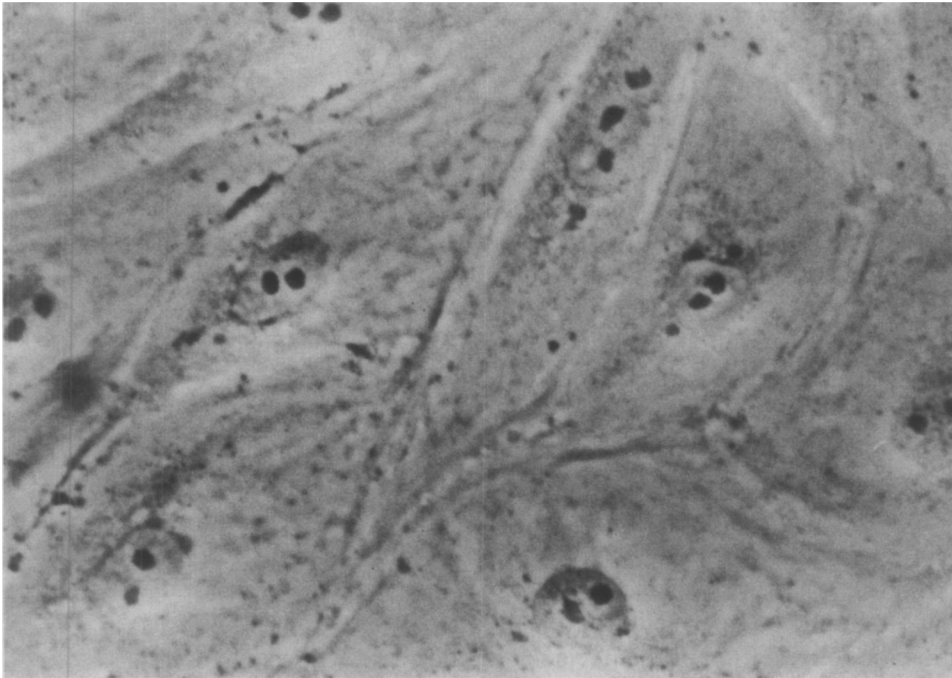


Fig. 1. Tetraploid PtK2 WA cells. $\times 220$.

chambers were opened in a sterile laminar flow hood and the non-irradiated cells were cleared away from the irradiated cell using a pneumatic micromanipulator [6, 7, 8]. The chamber was then placed in the CO₂ incubator at 37°C. The cells were monitored continuously under the light microscope. The killing of non-irradiated cells which migrated into the proximity of the cloned cells was performed by laser irradiation or micromanipulation. Trypsinization was used to remove the clonal descendants of the irradiated cell from the Rose chamber. The cells were transferred to a culture flask until thousands of subsequent daughter cells were produced.

Chromosome preparation. Cloned cells derived from irradiated cell were analysed cytochemically for the nucleolar organizer region by modified silver staining methods [12]. Mitotic dividing cells were blocked by treatment with 0.06 $\mu\text{g/ml}$ colcemid for 4–6 h, swollen with 0.075 KCl, fixed with acetic acid: methanol (3:1), dropped onto slides, and air-dried. A fresh solution of 0.5 g silver nitrate in 1 ml of distilled water was prepared. Drops of silver nitrate solution were placed on slides with good spreads of chromosomes, then a coverslip was placed on the slide, and kept in a 37°C incubator for 20 h. When the reaction attained the desired darkness, the slides were counter-stained with Giemsa stain, air-dried and mounted with Permunt. Chromosome spreads were located and photographed on a Zeiss photomicroscope with a 40 \times objective using Kodak high contrast copy film.

Table 1. Summary of results with PtK2 WA cells subjected to UV microbeam irradiation

Treatment of cell	Total no. of attempts	No additional mitosis or no formation of single nucleolus daughter cells	One additional mitosis, and 2 daughter cells with single nucleolus formed	More than one additional mitosis (cells with single nucleolus)	Successful clones
Irradiation of one nucleolar region	43	19	13	11	5

Results and Discussion

Irradiation of the PtK2 WA cells was usually conducted 3 days following seeding of the cells from culture flasks into Rose chamber. Appropriate cells with two nucleoli in early prophase were selected for irradiation. Early prophase was characterized by nuclear membrane breakdown, chromatin condensation, and nucleolar disappearance. UV laser beam was focused onto one of the two nucleoli through a 100× Zeiss ultrafluar objective to a spot diameter of approx. 0.25 μm. The cells irradiated were followed and observed continuously until they completed mitosis. Since irradiation of the nucleolus resulted in damage to the nucleolar DNA, resynthesis of only one nucleolus in each of the two post-mitotic daughter cells was observed. This result is similar to the results reported earlier [11].

As shown in table 1, the experiment was repeated 43 times. Two daughter cells, each deficient in one nucleolus, were produced 24 times (55.8% of the total cells studied). Of the 24 cases, 13 did not divide any further, and 11 continued through additional mitosis.

The isolation and cloning procedure was similar to that devised earlier for irradiated cells [6, 7, 8, 11]. When the clonal descendants of the original irradiated cells had reached confluency, the culture was subcultured until enough cells were available for freezing in liquid nitrogen. After establishment of the frozen stocks, the clonal cells still growing in the T25 flasks were analyzed further by karyotyping.

The cloning results were summarized in table 2. Six clones were not established as sublines and were lost because of mechanical or contamination problems during the cloning process. Five clones of irradiated cells maintaining the nucleolar deficiency were produced and established as sublines. The cloning efficiency of 69% was similar to those obtained in earlier studies involving chromosomal laser microbeam irradiation [6, 7, 8].

It was interesting to note that in the case of two clones, following several generations of cell division, some cells in the population had a normal nucleolus plus a small micronucleolus and other cells had only one nucleolus (fig. 2A–D). In addition it was observed that following further generations, some cells in the population returned to the normal two nucleolar conditions. The explanation of the initial recovery of one small nucleolus in subsequent daughter cells was

Table 2. Cloning results of laser-irradiated cells (PtK2 WA)

Treatment	No. of cases studied	No. of cases cloned	No. of cases failed to clone	Cloning efficiency (%)	Established sublines
Control (non-laser)	5	5	0	100	No attempts
Laser	16	11	5	69	5 ^a

^a The six lasered cells cloned, but not established as sublines, were lost because of mechanical and contamination problems.

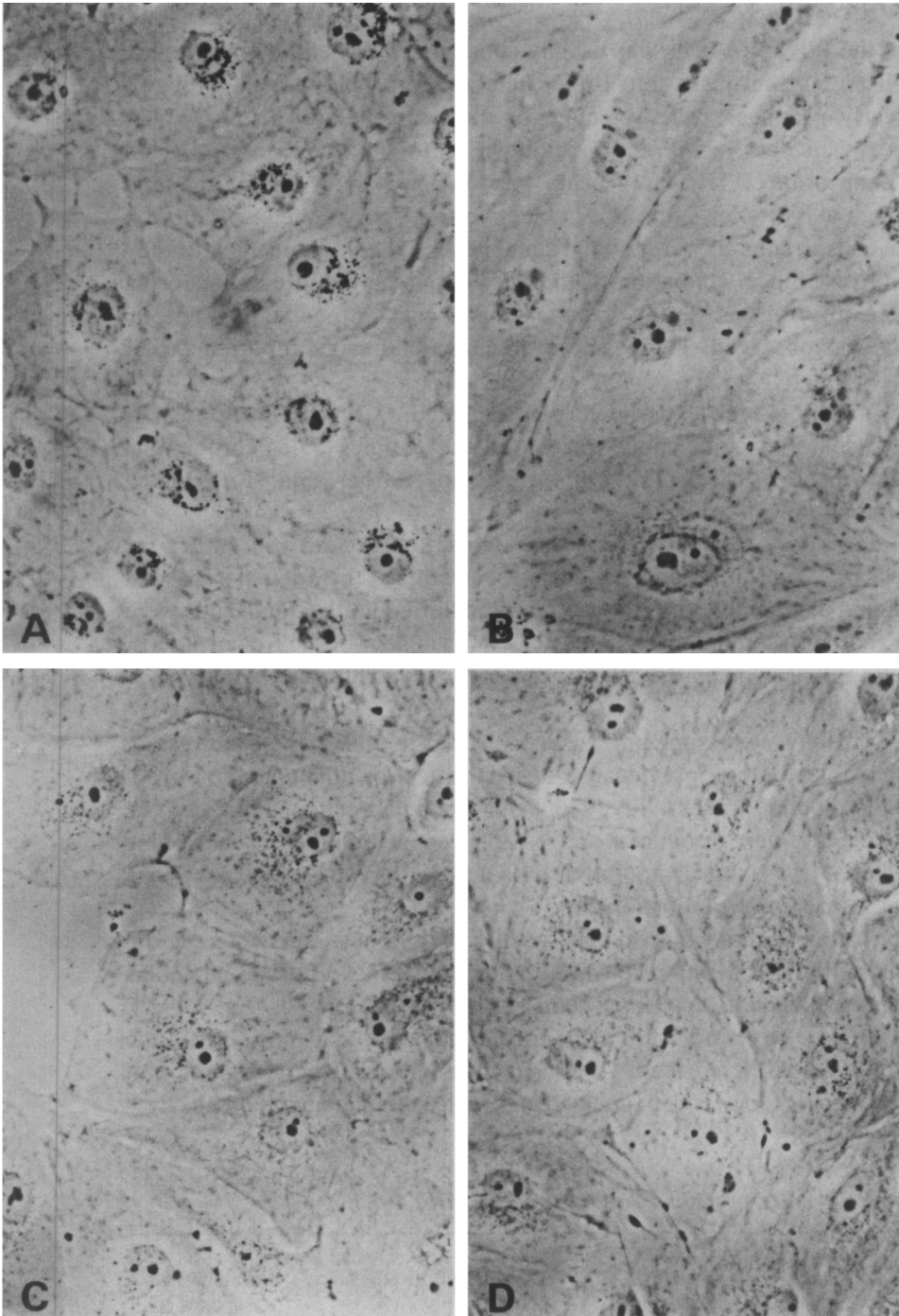


Fig. 2. (A) The population of cells with one nucleolus derived from the two daughter cells in clone No. 132. (B) Part of subsequent clonal cells with a normal nucleolus plus a small micronucleolus in clone No. 132. (C) The population of cells with one and two nucleoli derived from the two daughter cells in clone No. 115. (D) Part of subsequent clonal cells with a normal nucleolus plus a small micronucleolus in clone No. 115. $\times 120$.

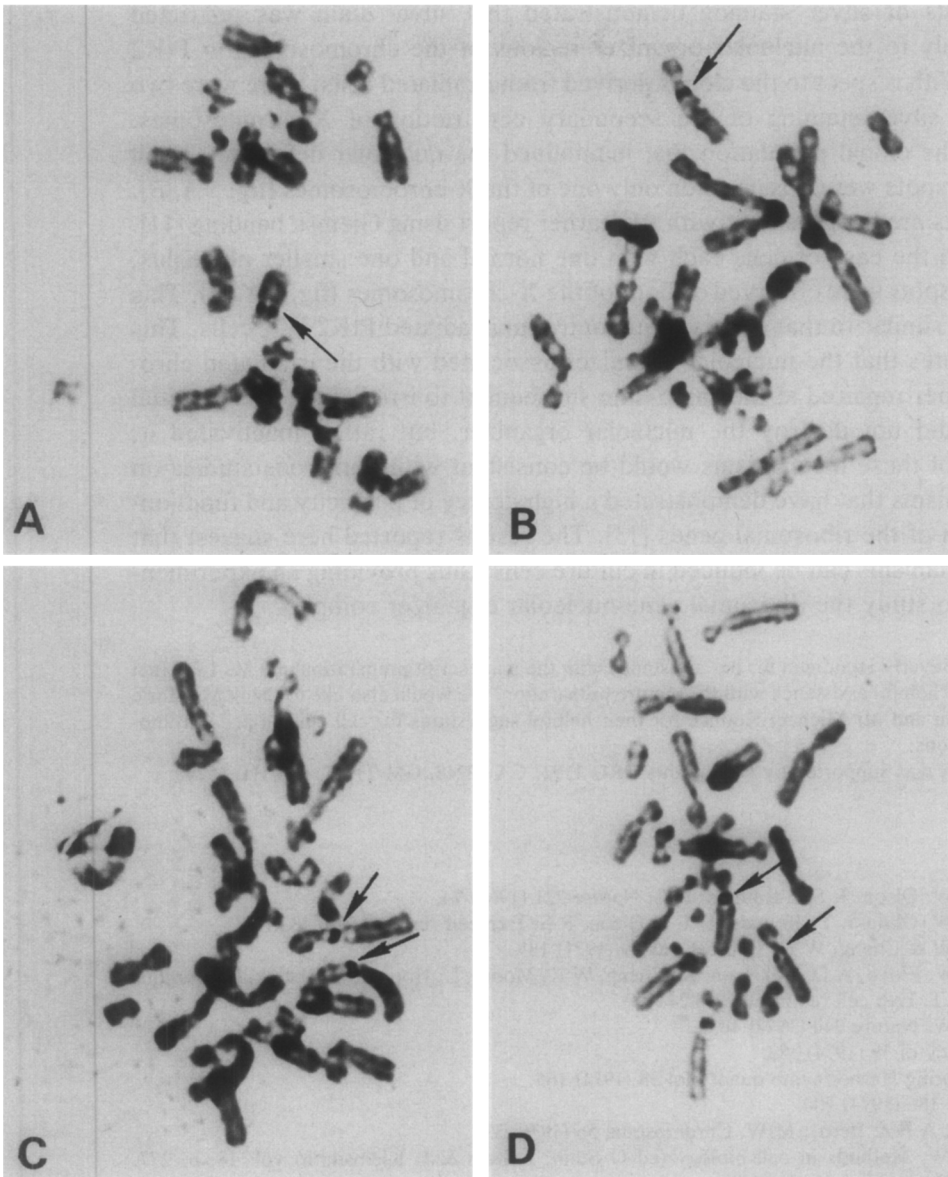


Fig. 3. Silver spots were observed on only one of the X chromosomes in cells with one nucleolus in (a) clone No. 132; (b) clone No. 115. Silver spots were observed on two X-chromosomes in cells with one normal nucleolus and one small micronucleolus in (c) clone No. 132; (d) clone No. 115. $\times 540$.

probably due to the fact that the damage following laser irradiation of one of the nucleolar regions was partially repaired after several generations, thus resulting in the synthesis of a small nucleolus. The damage was eventually totally repaired, thus resulting in a cell with two normal nucleoli. However, the fact that a significant proportion of the cells never returned to the two nucleolar conditions is interesting. Since all of the cells are cloned descendents of the original irradiated cells, it is surprising that some can correct the deficiency and others cannot.

The results of silver staining demonstrated that silver stain was restricted conspicuously to the nucleolar organizer regions of the chromosomes in PtK2 WA cells. With respect to the clones derived from irradiated cells, there were two patterns of silver staining of the secondary constriction of X-chromosomes. Firstly, in the clonal population that maintained the nucleolar deficiency in all cells, silver spots were observed on only one of the X-chromosomes (fig. 3 A, B). These results are in agreement with the earlier report using Giemsa banding [11]. Secondly, in the case of cells each with one normal and one smaller nucleolus, Ag-staining spots were observed on both of the X-chromosomes (fig. 3 A, D). This pattern was similar to that observed in control unirradiated PtK2 WA cells. This result indicates that the nucleolar organizer associated with the irradiated chromosome either repaired at the same time subsequent to irradiation, or the initial irradiation did not destroy the nucleolar organizer, but rather inactivated it. Either one of these mechanisms would be consistent with numerous studies on whole organisms that have demonstrated a high degree of plasticity and functional regulation of the ribosomal genes [13]. The results reported here suggest that similar mechanisms can be induced in culture cells, thus providing an experimental system to study the ribosomal gene-nucleolar organizer complex.

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References

1. Berns, M W, Olson, R S & Rounds, D E, *Nature* 221 (1969) 74.
2. Berns, M W, Ohnuki, Y, Rounds, D E & Olson, R S, *Exp cell res* 60 (1970) 133.
3. Berns, M W & Cheng, W K, *Exp cell res* 69 (1971) 185.
4. Berns, M W, Floyd, A D, Adkisson, K, Cheng, W K, Moore, L, Hoover, G, Ustick, K, Burgott, S & Osial, T, *Exp cell res* 75 (1972) 424.
5. Berns, M W, *Nature* 240 (1972) 483.
6. — *Int rev cytol* 39 (1974) 383.
7. — *Cold Spring Harbor symp quant biol* 38 (1974) 165.
8. — *Science* 186 (1974) 700.
9. — Branch, A B & Berns, M W, *Chromosoma* 56 (1976) 33.
10. Berns, M W, *Methods in cell biology* (ed G Stein, J Stein & L Kleinsmith) vol. 18, p. 277. Academic Press, New York (1978).
11. Berns, M W, Chong, L K, Hammer-Wilson, M, Miller, K & Siemens, A, *Chromosoma* 73 (1979) 1.
12. Goodpasture, C & Bloom, S E, *Chromosoma* 53 (1975) 37.
13. Tartof, K D, *Proc natl acad sci US* 71 (1974) 1272.

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