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## Nucleoli and Ploidy in *Potorous* Cells (PTK<sub>2</sub>) in vitro

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**Abstract.** Most cells of the male PTK<sub>2</sub> cell line contain one nucleolus, and they are diploid. However, a proportion of cells have more than one nucleolus. Cells with two and three nucleoli were isolated and cloned into viable populations. Greater than 90% of the cells in these clonal populations maintained the abnormal nucleolar number of the originally isolated cell. Karyotype analysis of cells with two and three nucleoli demonstrated that the cells were respectively tetraploid and hexaploid. It was concluded that in PTK<sub>2</sub> cells, nucleolarity is a good index of ploidy even if the ploidy level is abnormal. Furthermore, long term monitoring of the tetraploid cells demonstrated virtually no tendency towards reversion to the diploid condition as suggested by other studies in *Potorous*.

### Introduction

In most cells in vitro, the number of nucleoli per cell is quite variable (Phillips and Phillips, 1969; Alvarez-Lomeli, 1963; Gonzalez and Nardone, 1968). However, despite all the variations in nucleolar number between and within cells, there is considerable evidence to link the formation of each nucleolus with a nucleolar organizer region of the chromosome. This holds in both plants (Avanzi et al., 1972; Brady and Clutter, 1972) and animals (Macgregor and Kezer, 1973; M.L. Pardue, personal communication). Considerable amount of research on *Xenopus* has demonstrated a correlation of ribosomal DNA, secondary constrictions, and nucleoli. However, even with the apparent one-to-one relationship of nucleoli to secondary constrictions in *Xenopus*, there is considerable variability which suggests that the situation may not be so clear cut (Brown and Gurdon, 1964; Miller and Knowland, 1972). There is evidence in both plants and animals that certain nucleolar organizers may have dominance and result in the suppression of weaker ones (Brown and Blackler, 1972). Gerbi (1971) has investigated the possibility that sites of ribosomal (nucleolar) genes occur outside the secondary constriction in *Sciara corprophila*. In *Potorous* tissue

culture cells, it has been demonstrated that microirradiation of the nucleolar organizer site results either in the production of a compensatory nucleolus apparently from some other chromosomal region, or the production of numerous micronucleoli following cell division (Ohnuki et al., 1975; Berns et al., 1972).

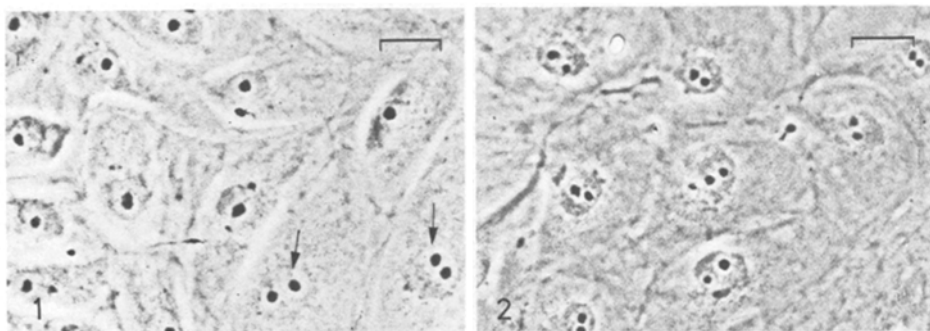
The data from the previous studies demonstrate that the number of nucleoli in a cell is variable and that this variability could be due to any number of causes. The studies reported here were undertaken in an attempt to further elucidate the causes for nucleolar variability. In particular, the existence of one secondary constriction and one nucleolus in most of the male *Potorous* cells suggested that these cells had a particularly stable nucleolar number in vitro. The occurrence of a small percentage of cells with a variable number of nucleoli provided the experimental material. Would these cells, if isolated and cloned, produce daughter cells with the same number of nucleoli? And, if so, what is the basis for this occurrence? Would it then be possible to establish clonal populations of cells with either a variable ploidy or a specific chromosomal content? And finally, what sort of pressure might there be to revert back to the normal number of nucleoli?

## Material and Methods

Rat kangaroo cells (PTK<sub>2</sub>) were obtained from the American Type Culture Collection and were grown as monolayer cultures in a modified Eagle's medium (Berns et al., 1972).

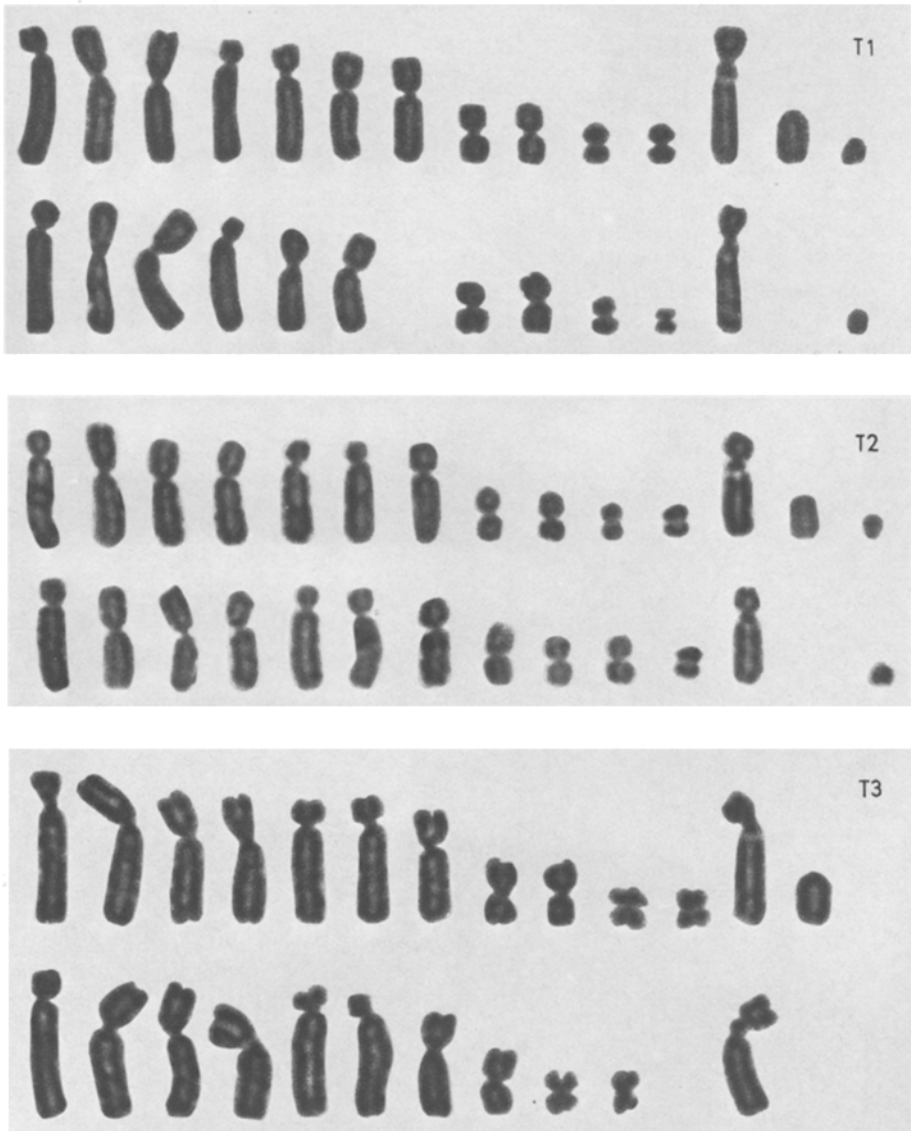
The cloning procedure was a variation of the one described earlier (Berns, 1974). Initially the clones were checked every day, and invading cells were removed either by repeating the cloning procedure or by placing the chamber under a laser microbeam system (Berns, 1974) and exposing the invading cells to multiple pulses of laser light. When the extent and speed of the PTK<sub>2</sub> migration was realized, however, cloned cells were either followed by time-lapse photography or checked every 5 h. When the clone contained 100–300 cells, it was transferred to a separate culture flask.

The karyotype procedure was the one described by Prescott (1964). Photographs were taken with a Zeiss photomicroscope using either phase or bright field optics.



**Fig. 1.** Normal population of PTK<sub>2</sub> cells exhibiting the typical single nucleolar morphology. Note the two cells (arrows) with two nucleoli; these cells are larger than the single nucleolar cells; scale = 20  $\mu$ m

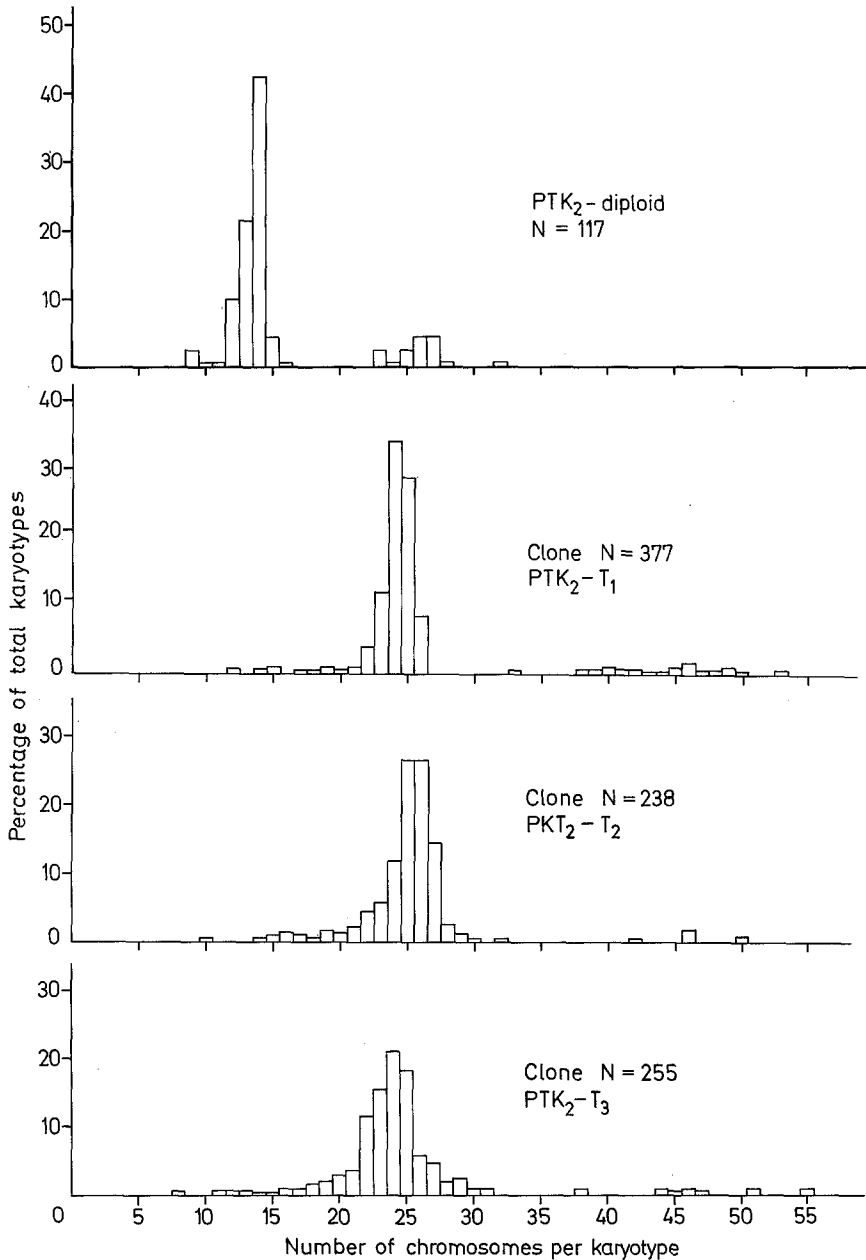
**Fig. 2.** Clonal population of PTK<sub>2</sub> formed from single isolated cells that had two nucleoli; scale = 20  $\mu$ m



**Fig. 3.** Karyotypes of the three double-nucleolar populations ( $T_1$ ,  $T_2$ ,  $T_3$ ). Note that there is some variability among the clonal sublines but that they are predominantly tetraploid

### Results

Although most PTK<sub>2</sub> cells possess a single nucleolus, between 2–10% of a normal population have two nucleoli (Fig. 1). Since double nucleolar PTK<sub>2</sub> cells repeatedly have been observed entering mitosis, cells with two nucleoli were found in three different Rose chambers and selectively cloned using micro-

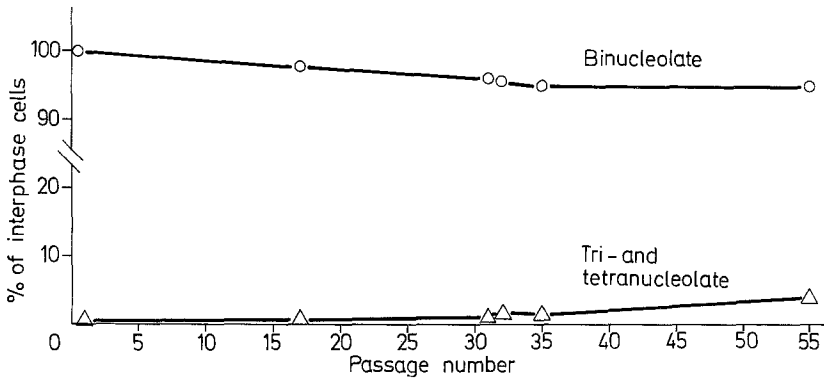


**Fig. 4.** Karyotype distribution data of  $T_1$ ,  $T_2$ , and  $T_3$  plus the parental diploid line

manipulation. The double nucleolar character of these cells proved heritable and has been maintained by all three clones for over a year. The cells in these clonal populations retain most of the features of the normal single nucleolar PTK<sub>2</sub> cells: they grow as an epitheloid monolayer and remain flat during mitosis

**Table 1.** Correlation of nucleolarity and ploidy in cell populations

Cell line	One nucleolus	2n	Two nucleoli	4n	Three-four nucleoli	> 4n
PTK <sub>2</sub> diploid	95.5%	83.3%	2.2%	15.4%	0.0%	0.85%
PTK <sub>2</sub> T <sub>1</sub>	1.2%	1.1%	95.8%	88.8%	1.5%	7.8%
PTK <sub>2</sub> T <sub>2</sub>	2.6%	1.8%	94.1%	87.6%	1.8%	2.1%
PTK <sub>2</sub> T <sub>3</sub>	2.3%	3.1%	92.7%	80.0%	3.6%	3.5%



**Fig. 5.** Maintenance of tetraploidy over 55 passages

(Fig. 2). However, when compared to the single nucleolar cells, they appear somewhat larger (Fig. 1).

Karyotype analysis indicates that those clones (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>) have chromosome numbers in the tetraploid range (Fig. 3). They contain 13–14 long autosomes and 7–8 short autosomes. Each appears to have two X chromosomes although both secondary constrictions were sometimes difficult to find. The number of Y chromosomes was subject to considerable variation. The parental population contained modal chromosome numbers of 7 long autosomes, 4 small autosomes, one X, and either one or two Y chromosomes. The major peak of total chromosomes for the parental population was 13–14. There was a minor peak with 24–25 chromosomes. The tetraploid cells responsible for this peak presumably give rise to the double nucleolar cells seen in culture. A comparison of chromosome distributions between the three tetraploid lines and the parental diploid line is presented in Figure 4.

When the percentage of a population having a particular number of nucleoli is compared to the percentage of karyotypes of a particular ploidy from that population, there appears to be a close correlation (Table 1). The percentage of single nucleolar cells is reasonably close to the percentage of diploid karyotypes, and the percentage of double nucleolar cells is similar to the percentage of tetraploid karyotypes.

Although the tetraploid clones appeared to be stable upon superficial inspection, this stability was more closely followed in clone T<sub>1</sub> (Fig. 5). Over a period

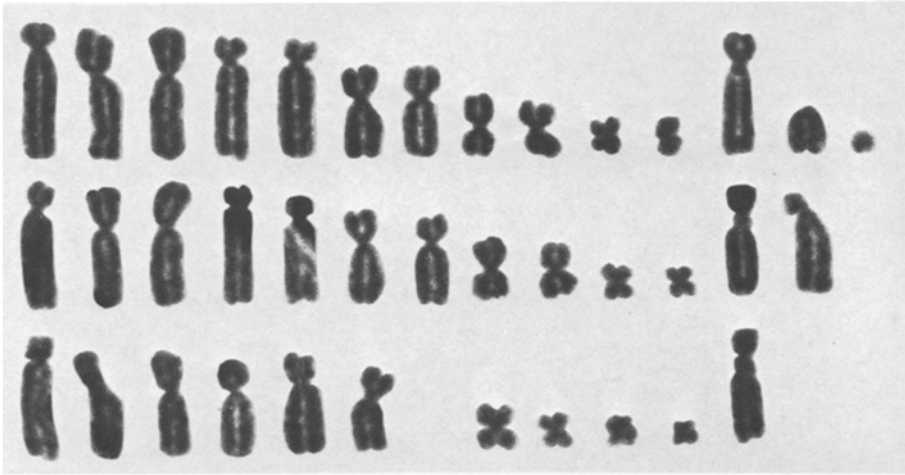


Fig. 6. Typical karyotype of three-nucleolar clone  $H_1$  indicating that it is hexaploid

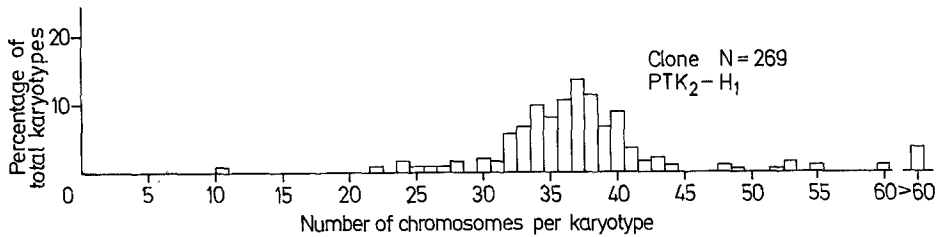


Fig. 7. Karyotype distribution of hexaploid clone  $H_1$

Table 2. Correlation of nucleolarity and ploidy in a hexaploid population ( $PTK_2 H_1$ )

One nucleolus	2n	Two nucleoli	4n	Three nucleoli	6n	Four nucleoli	8n
0.5%	0.4%	20.6%	5.6%	73.4%	80.7%	0.8%	6.3%

of 55 passages (roughly one passage/week), a slight reduction in double nucleolar (tetraploid) cells did occur. This reduction was associated with a rise in tri- and tetranucleolate cells. The proportion of single nucleolar cells was nearly constant.

In order to determine whether or not a further increase in nucleolar number is heritable and stable, an attempt was made to isolate and clone a three-nucleolar cell. This population was established as a subclone of tetraploid cell line  $T_3$ . The number of chromosomes per karyotypes in this clone was found to range from 11 to over 80. However, it had only one major peak and 80.7% of the karyotypes fell in the hexaploid range, containing between 33 and 45 chromosomes (Figs. 6, 7). As in the tetraploid clones, the number of nucleoli in these cells correlated well with the ploidy (Table 2).

## Discussion

A total of four clones were established from cells which had more than one nucleolus. Of these, three were predominantly double nucleolar and tetraploid; one had cells with predominantly three nucleoli and was hexaploid.

The percentage of double nucleolar cells in  $T_1$  was followed for over a year to determine the stability of the tetraploid population. The slight decrease was associated with an increase in the number of tri- and tetranucleolar cells. The percentage of cells with one nucleolus was nearly constant. This contrasts sharply with the results of Shaw and Krooth (1964) who studied primary cultures of rat kangaroo cells. At the second subculture, their population was almost 60% polyploid. By the fifth subculture, 75% of the karyotypes were in the diploid range. However, Martin and Sprague (1969) were able to establish relatively stable tetraploid populations from primary explants of human skin.

The correlation between ploidy and nucleolar number in these clones allows them to serve as tools for studying chromosome changes and mitosis. They let an experimenter compare the ploidy of parent and daughter cells by counting nucleoli. If this correlation holds true in  $PTK_1$  cells, which are female, this line should also be useful for studies of changes in ploidy.

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