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Nuclear Behaviour in Callus Cells: Morphology and Division

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Abstract. The cells of some pea, tobacco and haplopappus callus strains reveal considerable variability in nuclear morphology (polymorphous nuclei, differences in nucleolar size and number, enlarged chromocentres) and chromosome counts. The specific features in the nuclear morphology of callus cells are related with some pecularities in the reproduction activity of these cells (amplification, amitosis, fragmentation, various deviations from normal mitosis) under cultural conditions including both the definite action of the culture system and the absence of the regulatory control by the intact organism.

The karyologic instability of plant cells *in vitro* is a well-known fact. The variability of nuclear morphology and chromosome complement in plant tissue cultures has been described by a number of investigators (MITRA and STEWARD 1961, VENKETESWARAN 1963, MURASHIGE and NAKANO 1967, KALLAK 1968, DEMOISE and PARTANEN 1969, KAO *et al.* 1970, BAYLISS 1973, SUNDERLAND 1973, BOYER and SHANNON 1974, NOVÁK 1974, SALMIA 1974, SHAMINA and FROLOVA 1974, BROSSARD 1975, SINGH *et al.* 1975). Yet the literature on nuclear behaviour in tissue culture conditions is still insufficient, and we are rather ignorant of the fundamental causes.

The present study was undertaken to get some additional information on this subject by examining the nuclear morphology and division patterns in different callus strains.

Material and Methods

The callus tissues under investigation were derived from pea (*Pisum sativum* L. cv. 'Kiir') cotyledons, and from stem segments of tobacco (*Nicotiana tabacum* L.) and *Haplopappus gracilis* (NUTT.) GRAY.

All investigated callus strains belonged to the so-called "normal type" of undifferentiated callus tissues and were subcultured up to 8 years on a solid basal medium (haplopappus callus on Murashige and Skoog's, pea callus on Torrey's, and tobacco callus on White and Heller's medium) with appropriate supplements (2.4-D, adenine, IAA, kinetin, casein hydrolyzate, vitamins, and sucrose).

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The material for cytological observations was fixed in a 3 : 1 alcohol-acetic acid mixture, and squash preparations were stained by the aceto-orcein or Feulgen method.

Results

The investigated callus strains exhibited a remarkable diversity in nuclear size and form. Side by side with normal rounded or oval nuclei also lobed, fusiform, furrowed, fragmenting and other polymorphous nuclei of irregular outlines were found (Fig. 1).

In addition to the differences in nuclear size and form differences in nucleolar size and number were observed. Thus, in some callus cells nuclei with multiple or swollen nucleoli could be seen (Fig. 2).

Some callus cell nuclei were characterized by extraordinarily enlarged chromocentres (Fig. 3).

According to literature and the results of our previous investigations most callus cell nuclei divide by mitosis. Mitoses occur in diploid as well as polyploid nuclei. Yet several deviations from the normal course of mitosis could be observed. Thus, side by side with the normal regular arrangement of chromosomes at metaphase a segregation of chromosomes into separate groups was found (Fig. 4). Still more evident disturbances could be seen in the distribution of sister-chromatids at anaphase: either an unequal distribution took place or it was lacking altogether. A precise distribution of chromatids may be disturbed, for instance, in the case of bridges and fragments, resulting from certain aberrations in reproduction of chromosomes. Chromosomal bridges and fragments are very common phenomena in callus cell mitoses (Fig. 5). An inexact distribution of chromatids may also result from multipolar separation at anaphase (Fig. 6). The distribution of sister-chromatids into separate daughter-nuclei is wholly absent in the case of C-mitosis, wherein a complete spindle failure takes place and diplochromatids can be seen (Fig. 7).

Deviations from the normal cause of anaphase lead to variations on the ploidy level, either in the form of polyploidy or in the form of aneuploidy. Therefore, many callus strains are characterized by an instability of chromosome number.

Normally nuclear mitosis is followed by cytokinesis and two mononucleate daughter cells arise. The callus cells are frequently multinucleate ones, evidencing the failure of cytokinesis (Fig. 8).

In all callus strains under consideration amitotic nuclear figures were found (Fig. 9). In addition, nuclear division in the form of fragmentation could be seen in some callus cells (Fig. 10).

Discussion

It may be suggested that different pecularities observed in the nuclear morphology and division of callus cells are interrelated one with another and can be traced back to special reproduction patterns of these cells.

Although reproduction is a fundamental characteristic of life, the purpose of reproduction processes is not always the same, and, therefore, the course and the outcome of reproduction may be different in different biosystems or under different conditions. In intact biosystems reproduction processse are

ordinarily subjected to rather strict control: the reproduction of certain structures occurs at a definite place and at a definite time, whereas the duplication is normally followed by a precise distribution and spatial separation of the duplicated structures. Thus, in meristematic cells in which the main purpose of reproduction activity is the production of genetically equivalent daughter cells, we can see a complete sequence of duplication and separation patterns, from DNA molecules to daughter cells (or the so-called normal cell cycle, including G_1, S, G_2 and mitosis). The total duplication of DNA is followed by the separation of sister-molecules into sister-chromatids, the duplication of chromatids — by their separation into daughter nuclei, and the duplication of nuclei — by their separation into daughter cells.

In many differentiated tissue cells as well as in callus cells reproduction processes evidently serve somewhat different purposes. That is why the regular cycle of duplication and separation patterns, characteristic of meristematic cells, may stop or alter at any stage.

Thus, DNA replication may be limited to duplication of single gene loci. If the extra replication of certain DNA sequences occurs in heterochromatin, it results in the formation of enlarged chromocentres (NAGL 1972). There are also some reports that extra DNA synthesis is related to nuclear fragmentation (NUTI RONCHI *et al.* 1974). If the chain of reproduction processes breaks off immediately after the total DNA replication, without being followed by separation of duplicated chromonemata, it leads to the formation of polytenic chromosomes. If the reproduction cycle stops at early prophase, before the onset of chromosomal coiling, endomitosis takes place and produces an enlarged polyploid nucleus. If the reproduction cycle breaks off at metaphase, C-mitosis occurs and also leads to polyploidy. Besides, various deviations from the normal course of mitosis may happen, resulting in a variability of chromosome counts and nuclear morphology. The segregation of chromosomes at prophase or metaphase, as well as chromosome bridges at telophase may produce various polymorphous nuclei, among them constricted amitotic ones. Finally, the cessation of the reproduction cycle directly after telophase (without being followed by cytokinesis) leads to the formation of multinucleate cells.

Commonly, the course of mitosis and cytokinesis in meristematic cells is regarded as a standard of the reproduction cycle at plant cell level. Therefore, all deviations from such a course of reproduction are considered as "pathological" and "abnormal". Actually, in certain conditions these "abnormalities" express a normal state of the cell cycle. This statement is evidenced by the fact that various deviations from normal mitosis as well as variations in nuclear morphology do not appear only in callus or tumor cells or in other "pathological" cell cycles, but they have been reported to occur in normal tissue cells of intact plants, although with a much lower frequency (GOLDSTEIN 1928, COLEMAN 1950, CZEIKA 1956, TSCHERMAK-WOESS 1956, D'AMATO 1964, PARTANEN 1965, NAGL 1970, 1973, REESE 1973, SEN and BHADURI 1969).

The higher frequency of various deviations from the complete course of mitosis in callus cells can be explained by the special conditions of culturing, including both the lack of regulatory control by the intact organism and the definite action of the culture system. The latter, in turn, operates as a set of factors which take part in the realization of genotypic information, act as

mutagens and/or have a selective effect on the heterogeneous callus cell population.

As a matter of fact, the metabolic activity of plant cells in culture increases. Both the enlarged chromocentres and nucleoli, the enlargement of nuclear surface in polymorphous nuclei and polynucleate cells can be conceived as cytological evidence of an increased metabolic activity. On the other hand, the nuclear division by fragmentation or amitosis does not lead to such structural and functional reorganizations or interruptions as mitosis does. Therefore, both the nuclear fragmentation and amitosis may be regarded as a normal step in the callus cell development (NUTI RONCHI *et al.* 1973). It has recently been reported by W. Nagl that alterations in cell cycle course are related to the action of growth regulators (NAGL 1974).

Besides, the culture system obviously induces some changes in the genetic material of callus cells. Thus, certain chemical constituents of the medium (such as auxins and other biologically active substances) may act as mutagens and increase the mutation rate, including the chromosomal one (KALLAK and YARVEKYLG 1971).

Finally, one has to consider the fact that cultural conditions may operate as selective factors, differentially promoting the maintenance and reproduction of genetically heterogeneous callus cells. The frequency of heteroploid cells, and that of various nuclear division patterns (including different deviations) will depend on the selective effects of the culture system. Moreover, there may be competition between genetically heterogeneous cells resulting in the establishment of definite callus cell lines.

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Figures at the end of the issue.

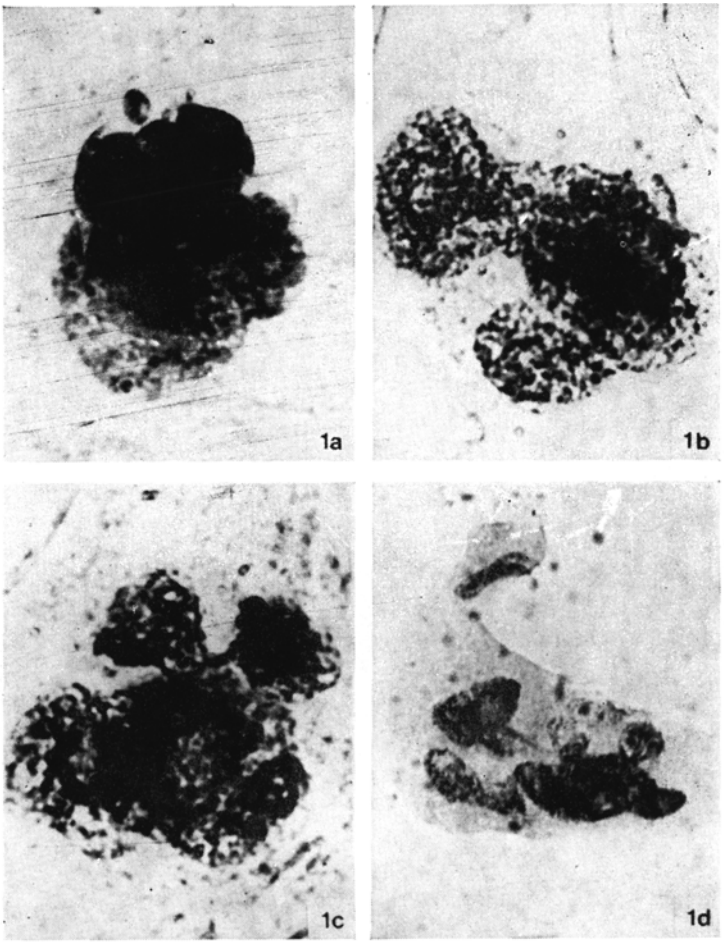

Fig. 1. Polymorphous nuclei. (a), (b) in tobacco callus cells. ×1750; (c) in pea callus cell. ×1750; (d) in pea callus cell. ×920.

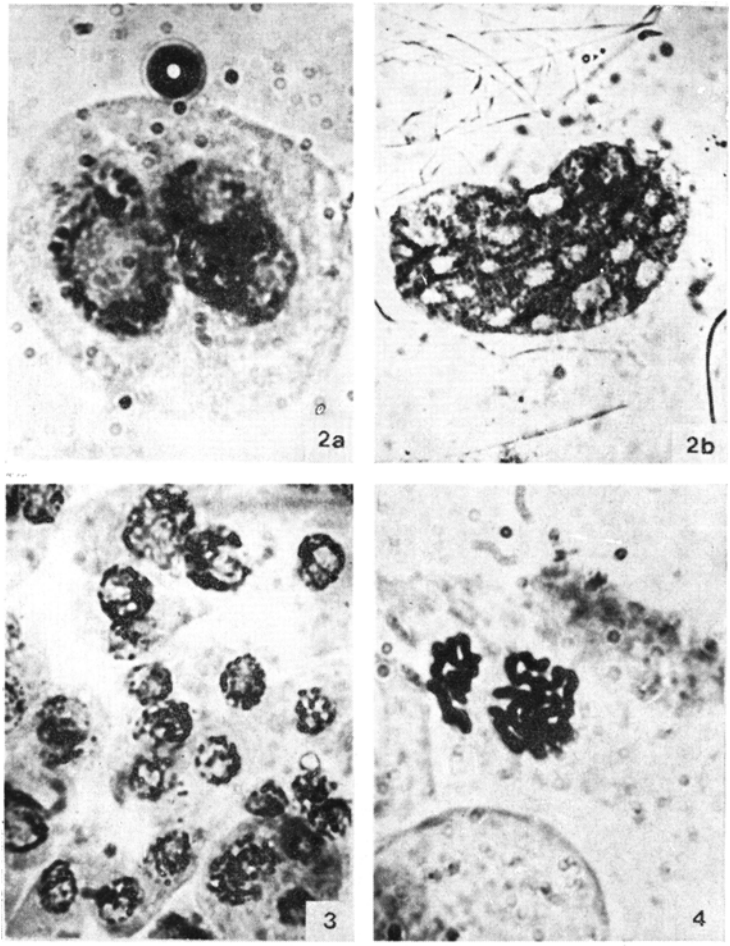

Fig. 2. Nucleoli. (a) swollen nucleoli in binucleate pea callus cell. ×1750; (b) multiple nucleoli in enlarged pea cell nucleus. ×920.
Fig. 3. Enlarged chromocentres in pea cell nuclei. ×920.
Fig. 4. Segregation of chromosome at metaphase in pea callus cell. ×1750.

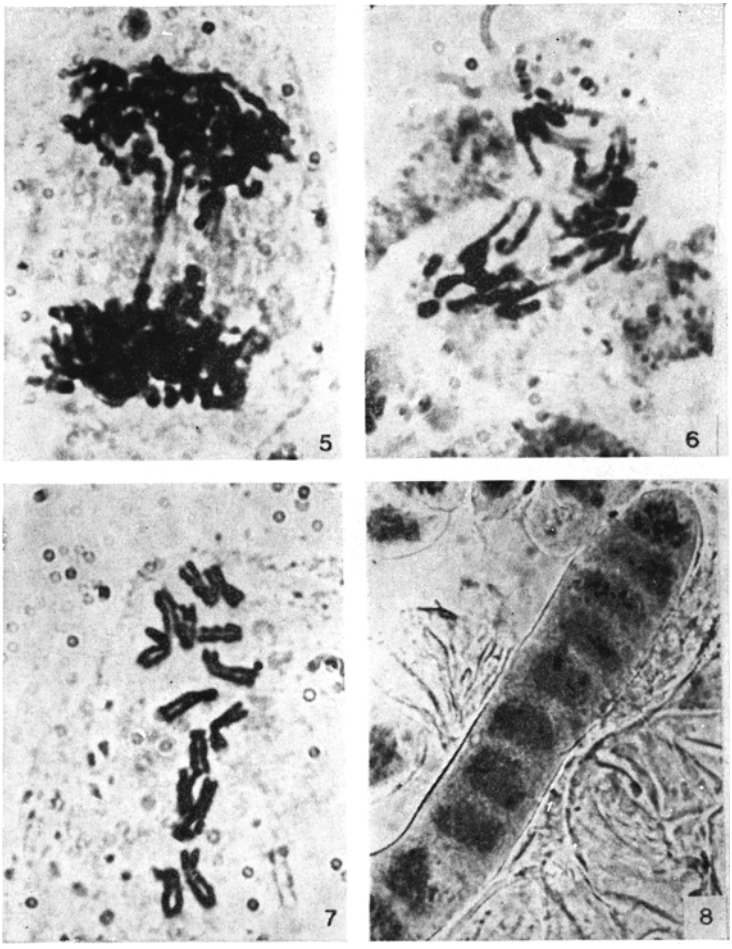

Fig. 5. Aberrant telophase in pea callus cell. ×1750.
Fig. 6. Multipolar anaphase in pea callus cell. ×1750.
Fig. 7. C-mitosis in pea callus cell. ×1750.
Fig. 8. Multinucleate pea callus cell. ×920.

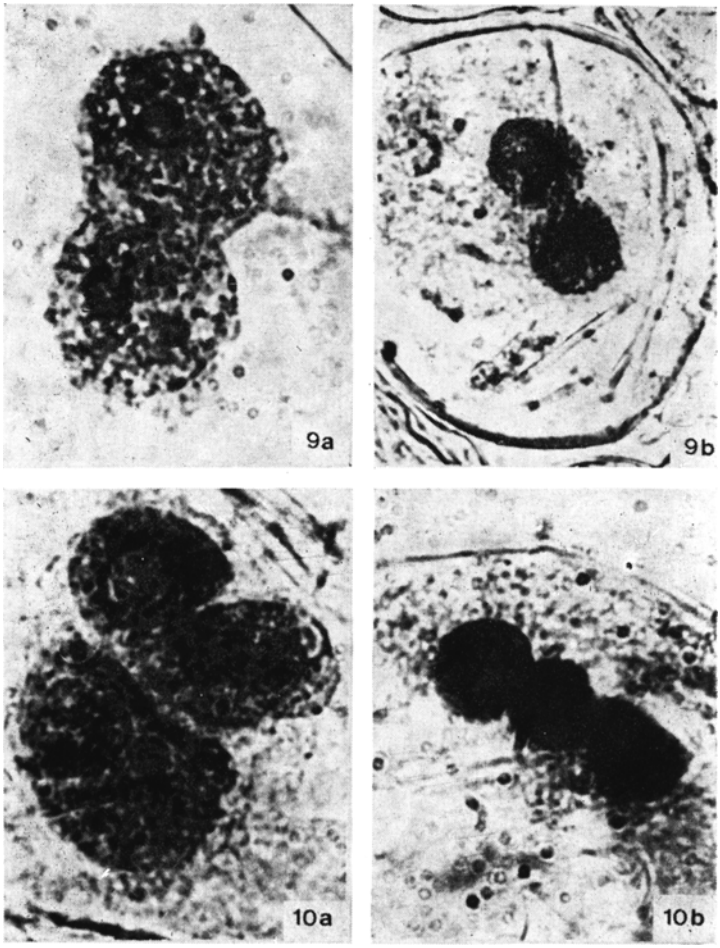

Fig. 9. Amitotic nuclear figures. (a) in tobacco callus cell; (b) in pea callus cell. ×1750.
Fig. 10. Nuclear fragmentation. (a) in tobacco callus cell; (b) in pea callus cell. ×1750.