SOME OBSERVATIONS ON THE BEHAVIOUR OF COLCHICINE-TREATED CELLS DURING RECOVERY IN ALLIUM CEPA*

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ABSTRACT

The accidental discovery of a cell with haploid number of diplochromosomes in squashes of colchicine-treated root tips led to a search for haploid anaphases in treated roots allowed to recover in water. No haploid anaphases were seen. Apart from the divisional stages of diploid and tetraploid nuclei, cells with two pro-, meta- and ana-phases were observed. The formation of distinct cell boundaries by each nucleus of an originally multinucleate cell indicates their potentialities in this direction.

INTRODUCTION

The phenomenon variously termed as somatic meiosis, somatic reduction, reductional groupings and reductional type mitosis, has been extensively discussed by several investigators (Grell, 1946; Huskins, 1948 a and b; Wilson and Cheng, 1949; Huskins and Chouinard, 1949; Huskins and Cheng, 1950; Allen, Wilson and Powell, 1950; Battaglia, 1950; Wilson, Hawthorne and Tsou, 1951; Lindahl, 1953 a and b; Sharma and Sen, 1954; Berger, McMahon and Witkus, 1955; Sharma and Datta, 1956, 1959; Srinivasachar and Patau, 1958; De Robertis, Nowinski and Saez, 1960; Swanson, 1960; Bouharmont, 1961). The crucial evidence for such claims is the rare occurrence of cells with the haploid or subhaploid complement of chromosomes in normal material (Srinivasachar, 1958; Royan-Subramaniam, 1964). The discovery of a single cell having a haploid number of diplochromosomes in haematoxylin squashes made immediately on termination of exposure to colchicine (Subramanyam, 1964) led to an exploration whether the anaphases

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of such haploid cells could be located by allowing the colchicine-treated roots to recover in distilled water.

**Material and Methods**

Roots of freshly germinated bulbs of *Allium cepa* were kept immersed in a 0.2% colchicine solution overnight (18½ hours). Some of the roots were fixed in acetic alcohol for 2 and 24 hours on termination of colchicine treatment while others were allowed to recover in distilled water and fixed, after 24, 48, 72 and 96 hours after such transfer in acetic alcohol or for one hour in Iodine-Formal-Acetic acid (Subramanyam, 1960). The roots fixed for 2-24 hours in acetic alcohol and one hour in Iodine-Formal-Acetic acid were hydrolysed in N HCl at 60°C for 6-8 min. and 10 min. respectively, were mordanted for 10-20 min. in 4% ferric ammonium sulphate, washed for 10-15 min. in two to three changes of distilled water and stained with Heidenhain's haematoxylin for 15 min. They were squashed by the haematoxylin squash technique described earlier (Subramaniam and Subramanyam, 1961). Observations were made from temporary mounts, photographed and made permanent by the tertiary butyl alcohol method (Royan, 1961). Some of the preparations have been photographed with the phase contrast microscope to accentuate the delineation of the cell boundaries and other details.

**Observations**

*Immediately after treatment.*—The inactivation of the spindle and the consequent irregular scattering of the chromosomes with the resultant formation of two or more nuclei in the cell is illustrated in Photos 1 and 2. While the nuclei are almost similar in size in Photo 1, they exhibit a range in size in the multinucleate cell shown in Photo 2. It was shown earlier that in such colchicine-treated material a cell possessed a haploid complement of diplochromosomes (see Photos 5 and 6, Subramanyam, 1964). This led to a search for haploid anaphases in root tip cells allowed to recover in distilled water following colchicine treatment.

*On recovery in distilled water.*—The maximum number of divisional figures was observed 24 hours after transfer to distilled water. They were few after 48 hours and excepting for very rare instances of 4 n metaphases with highly contracted chromosomes after 48 and 72 hours most of the cells were in the interphase condition after 72 and 96 hours. This gradual reduction in the number of divisional phases with passage of time during recovery was observed by Berger and Witkus (1962) in roots treated with 5-fluorouracil,
Colchicine Treated Cells During Recovery in Allium cepa

In squashes of roots allowed to recover for 24 hours many normal pro- (Photo 3), meta- (Photo 4) and ana-phases (Photo 5) were observed. Though identical stages of tetraploid cells were also observed (Photos 6-10) their percentage was only of the order of 2% judged from a count of 1,273 cells from 27 fields.

Apart from the normal stages of mitosis of the $2n$ and $4n$ cells described earlier after a 24-hour recovery, certain other configurations were also observed. The presence of two pro- and meta-phases in a single cell is exemplified by Photos 11 and 12 (see also Bouharmont, 1961). When these pass into the anaphase they may give the orientation of chromosome groups exemplified by photos 13 and 14. Whether the two daughter anaphase groups lying close together in the middle of the cell in Photo 13 may during late ana- or early telo-phase get clumped together into a mass as in Photo 14 is a possibility to be envisaged. The final result of the presence of such double anaphases in cells would be the reconstitution of two diploid and one tetraploid nucleus. Uni- but bilobed and multi-nucleate cells obtained after 24 and 48 hours of recovery are shown in Photos 15 to 20.

Cells with lobed nuclei of the type shown in Photos 15 to 17 are possible of derivation from the orientation of two daughter anaphase groups in the middle of the cell as in Photo 13. The division of a multinucleate cell into several uninucleate ones by the formation of distinct cell boundaries is shown in Photos 19 and 20 (see also Heneen, 1963).

**DISCUSSION**

It was shown earlier (Subramanyam, 1964) that the occurrence of diplochromosomes could be only due to a replication of the chromosomes following the formation of the haploid cell and was therefore indicative of the origin of rare viable haploid cells from reductional groupings (see also Srinivasachar and Patau, 1958; Royan-Subramaniam, 1964).

Haploid anaphases were not observed during recovery in treated roots when the preparations were scanned. There was a doubt, however, whether each meta- and ana-phase group in Photos 12, 13 and 14 had the complete diploid complement. The inability to count the actual number leaves the question open. Disorganization of the spindle by colchicine implies as a corollary the inability of those cells in anaphase at the time of exposure to colchicine to form cell walls. When such binucleate cells come into mitotic phase during recovery the possibility of orientation of two neighbouring daughter anaphase groups in the middle of the cell (Photos
13 and 14) has to be envisaged. The result should be the formation of a tetraploid nucleus by the two daughter anaphase groups lying near each other in the middle of a cell.

Micronuclei, possessing as they do a subdiploid chromosome number, are generally believed to disintegrate because of their non-viability. The initiation of cell boundaries by each nucleus of an originally multinucleate cell indicated the potentialities in this direction of each nucleus though not the viability of the cells so formed (Photos 19 and 20).

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REFERENCES


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EXPLANATION OF PLATE VI

PHOTO 1. A binucleate cell, × ca. 900.
PHOTO 2. A multinucleate cell with nuclei of differing sizes, × ca. 800.
PHOTOS 3-5. Normal pro-, meta- and ana-phases.
PHOTO 6. Tetraploid prophase.
PHOTOS 7 & 8. Tetraploid metaphases. Note the two SAT-chromosomes with stumpy grains projecting out at the right end of the group in Photo 8.
PHOTOS 9 & 10. Tetraploid anaphases. The SAT-fibres with grains at their tips are seen projecting out from the middle of the upper group in Photo 10.
PHOTOS 11 & 12. Two pro- and meta-phases found in single cells, × ca. 550.
PHOTOS 13 & 14. Double anaphases located linearly in single cells. Two daughter groups are close to each other in the middle of the cell in Photo 14, × ca. 550.
PHOTOS 15-17. Uninucleate cells with differing bilobed configurations, × ca. 550.
PHOTOS 18-20. Multinucleate cells. Note the formation of distinct cell boundaries around the nuclei in Photos 19 and 20. Photo 18, × ca. 350 Photos 19 and 20, × ca. 1,050.

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