ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 56, nr 4: 599-610
1987

Translocation of nuclei and reorientation of the mitotic apparatus in the ontogeny of stomata in *Tradescantia virginiana* L.

JADWIGA A. TARKOWSKA, MIROSŁAWA WAŻYŃSKA, ALINA A. JABŁONOWSKA

Department of Plant Anatomy and Cytology, Warsaw University,
Krakowskie Przedmieście 26/28, 00-927 Warsaw, Poland
(Received: January 27, 1987. Accepted: March 31, 1987)

Abstract

In vitro studies of the ontogeny of stomata of young leaves of *Tradescantia virginiana* L. showed that: 1) translocation of nuclei, often along long, winding paths took place in companion cell mother cells before the onset of asymmetric mitosis, 2) the reorientation of the entire mitotic apparatus took place during division of quard cell mother cells. Pertinent factors which may play a role in both processes are indicated.

Key words: stomata, nuclear translocation, reorientation of the mitotic apparatus

INTRODUCTION

Stomata are the subject of numerous investigations dealing with their ontogeny as well the structure of the mature stoma and its physiological functions. A stoma is formed by 2 quard cells, with a pore (aperture) between them and subsidiary (accessory) cells which usually surround it, and whose number and origin can be varying.

In 1970, Kaufman et al. directed attention to the fact that in *Avena sativa*, asymmetric mitoses leading to the formation of subsidiary cells are preceded by the translocation of nuclei inside these cells.

Another interesting phenomenon found by Palevitz and Hepler (1974a) and Palevitz (1986) in *Allium cepa* cotyledons is the change in the plane of division (reorientation) of quard cell mother cells (GCMC). The authors

observed typical tropometaphases in vitro and in the electron microscope (EM). During anaphase and telephase, the entire mitotic apparatus (including the cell plate which is being laid down) is reoriented so that the two sister cells, separated by a cell wall, are oriented along the long axis of the leaf.

Translocation of nuclei and reorientation of the mitotic apparatus are the subject of the investigations presented in this paper.

MATERIAL AND METHODS

The lower epidermis of very young leaves of *Tradescantia virginiana* L. (*Commelinaceae*) was studied. The material was collected from plants growing in an experimental garden or in pots in a greenhouse. In vitro observations were made on hand-cut, very thin sections, tangent to the surface, and on fragments of epidermis torn off of the abaxial surface of the basal part of the leaf.

The slides were viewed and photographed in a Zetopan (Reichert) phase contrast microscope in a drop of 2% aqueous glucose in order to keep the cells alive longer.

RESULTS

Tradescantia virginiana L. leaves are amphistomatic with more numerous stomata on the bottom (abaxial) side. The stomata are irregularly dispersed, but oriented along the long axis of the leaf. A mature stoma (tetraperigenic according to the classification of Fryns-Claessens and Van Cotthem 1973), is formed by 2 quard cells and 4 subsidiary cells: 2 smaller lateral ones and 2 larger polar cells (Fig. 1).

The meristemoid (the mother cell of the quard cell — GCMC) differentiated as the result of unequal division of an epidermal cell (Fig. 3). Next, 2 lateral subsidiary cells were formed through the division of 2 epidermal cells, located on both sides of the meristemoid. These divisions occurred either successively (Fig. 7) or simultaneously. In a similar way, 2 polar subsidiary cells were formed either successively (Figs. 8-12) or simultaneously (Fig. 7). All 4 of the mitoses in the epidermal cells surrounding the meristemoid were always asymmetric.

The subsidiary cell mother cells were large, highly vacuolized. Large nuclei, suspended on cytoplasmic bridges, were usually positioned near the center of the cell, although when the nucleus entered into mitosis, as a rule, it was in a parietal position (near the GCMC) (Figs. 7, 8). As a result of this division, a smaller subsidiary cell was formed and the larger, sister cell remained an epidermal cell. Before this unequal, in other words asymmetric,

division, the nucleus had been translocated, changing its position in the cell several times. Because of the shape and arrangement of the epidermal and GCMC cells, the observations on the motion of nuclei were concentrated on the polar cell mother cells (PCMC), in which the nuclei have more space and their motion is more distinct. Very numerous in vitro observations showed that the nucleus moved from its initial, central position in the cell, sometimes to the furthest possible position, that is to cell wall opposite to the meristemoid (GCMC), and then returned to its premitotic position (near the GCMC). This was the shortest path that the nucleus covered. It was amazing to find that the nucleus often wandered along long and winding paths, as shown on Figs. 13-18. It sometimes wandered from one long wall to the opposite one, "bounced off" from it, moved to the distant pole and then returned to the vicinity of the GCMC. The duration of the movement of the nucleus in the cell was variable, depending on the length of its path. Under the conditions of the experiment, the shortest path (to the pole opposite the GCMC and back to the place of division) lasted up to 5 hours.

Simultaneously with the nuclear translocations, the length of cytoplasmic bridges also size and arrangement of vacuoles clearly changed (Figs. 13-18). Division of the nucleus was usually begun and completed near the GCMC, although sometimes (especially when the nucleus covered a longer path) it began almost in the center of the cell and during mitosis, the entire mitotic apparatus was translocated towards the GCMC (Figs. 9, 10). In both cases the divisions were asymmetric. The cell plate developed centrifugally (Fig. 12) and this process lasted around 3 hours (under the conditions of the experiment). Initially, the plate was slightly arched, then it flattened and the new cell wall was formed parallel to that of the meristemoid.

After the formation of the subsidiary cells, the GCMC grew, from an isodiametric cell, became more elongate and began to prepare for division. The division of its very large, centrally positioned nucleus (Figs. 10, 11), and subsequent cytokinesis, led to the formation of 2 quard cells. The mitosis in progress was typically tropokinetic, which was most clearly seen at metaphase. In anaphase, the interzonal area ran initially almost obliquely across the cell, but from late anaphase and in telophase, the centrifugally developing cell plate and daughter nuclei gradually changed their position from oblique to vertical, that is, in agreement with the longer axis of the cell and leaf (Figs. 19-24). The reorientation of the plane of division lasted in this species from 40 to 60 minutes. In a few cases, the separation of chromosomes in anaphase took place perpendicularly to the long axis of the leaf and did not require the later change in position of the daughter nuclei and developing cell wall.

It was also possible to observe the reorientation of the mitotic apparatus in the earliest stages of differentiation of the stoma, that is, during the

PLATE I

Surface sections from the abaxial epidermis of a leaf, in vitro, phase contrast (with the exception of Figs. 2 and 3)

Fig. 1. A mature stoma. Arrow — stoma, GC — quard cells, SC — lateral subsidiary cells, PC — polar subsidiary cells, ×6000. Figs. 2 and 3. Mitoses in epidermal cells, acetoorcein. Long arrows — tropokineses, short arrow — GCMC, asterisk — unequal division, but the proper plane of division, ×900. Figs. 4-6. Asymmetric, oblique divisions in epidermal cells, GCMCs will probably develop from the lower, smaller cells. The centrifugally developing cell plate — arrow, ×1200

PLATE II

Surface sections from the abaxial epidermis of a leaf, phase contrast

Fig. 7. Simultaneous prophases in two PCMCs and in one SCMC, one subsidiary lateral cell is already present — arrow, ×600. Fig. 8. Prophase in a PCMC, the nucleus is adjacent to the GCMC cell wall, ×900. Figs. 9 and 10. Late prophase (Fig. 9) and prometaphase (Fig. 10). The dividing nucleus is suspended on cytoplasmic bridges in the center of the cell, ×900. Figs. 11 and 12. Polarized telophases in PCMCs, cell plate is developing centrifugally, ×900

PLATE III

Surface sections from the abaxial epidermis of a leaf, in vitro, phase contrast

Figs. 13-18. Translocation of the nucleus in an abaxial PCMC. Changes in the vacuolization (v) and length of cytoplasmic bridges (arrows), ×1000

PLATE IV

Surface sections from the abaxial epidermis of a leaf, in vitro, phase contrast

Figs. 19-24. Reorientation of the mitotic apparatus in a dividing GCMC. The centrifugally developing cell plate — arrows, ×1600

differentiation of GCMC. Various planes of division were found in the very young, growing epidermis. In some cases, the spindle axis were oriented along the long axis of the leaf, and the daughter cells, of equal size, remained epidermal cells (Fig. 2). In another cases, a tropokinetic division took place (Figs. 2, 4). The oblique orientation of the mitotic apparatus was visible from metaphase on. The developing cell plate was also oblique (Fig. 5), but during development underwent reorientation and the young cell wall was parallel to the long axis of the leaf (Figs. 2, 6). The result of such a division was the formation of two cells, usually of unequal size, of which the smaller usually became the GCMC, while the larger remained an epidermal cell, capable of further divisions. The results of these observations are not unequivocal, since the formation of all of the GCMCs was not always linked with reorientation, and not every tropokinesis (and reorientation) led to the development of GCMC (Fig. 3).

After completion of divisions, the 6-celled stoma assumed its final form: the subsidiary cells grew, the quard cells changed their shape (from elongate

PLATE I

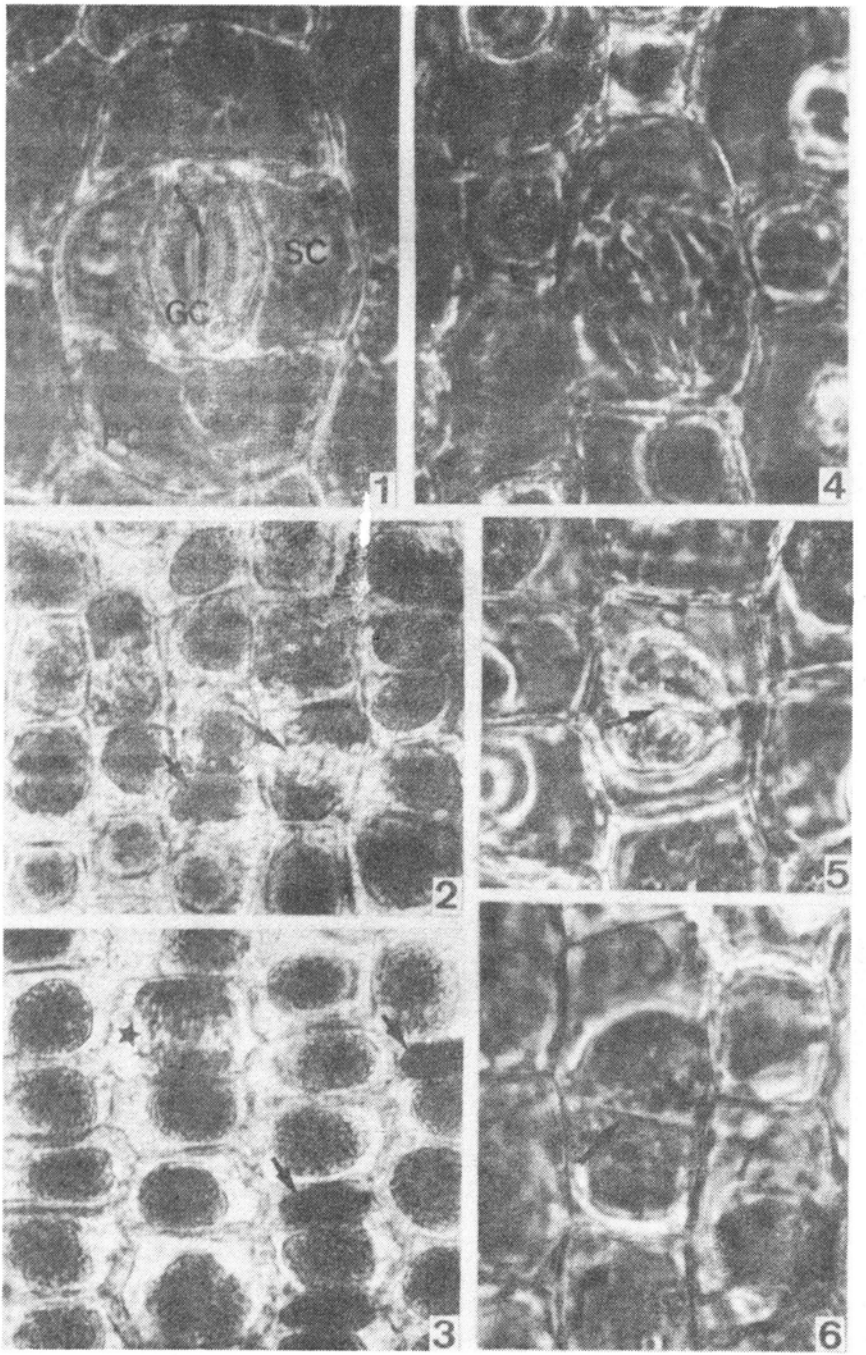


PLATE II

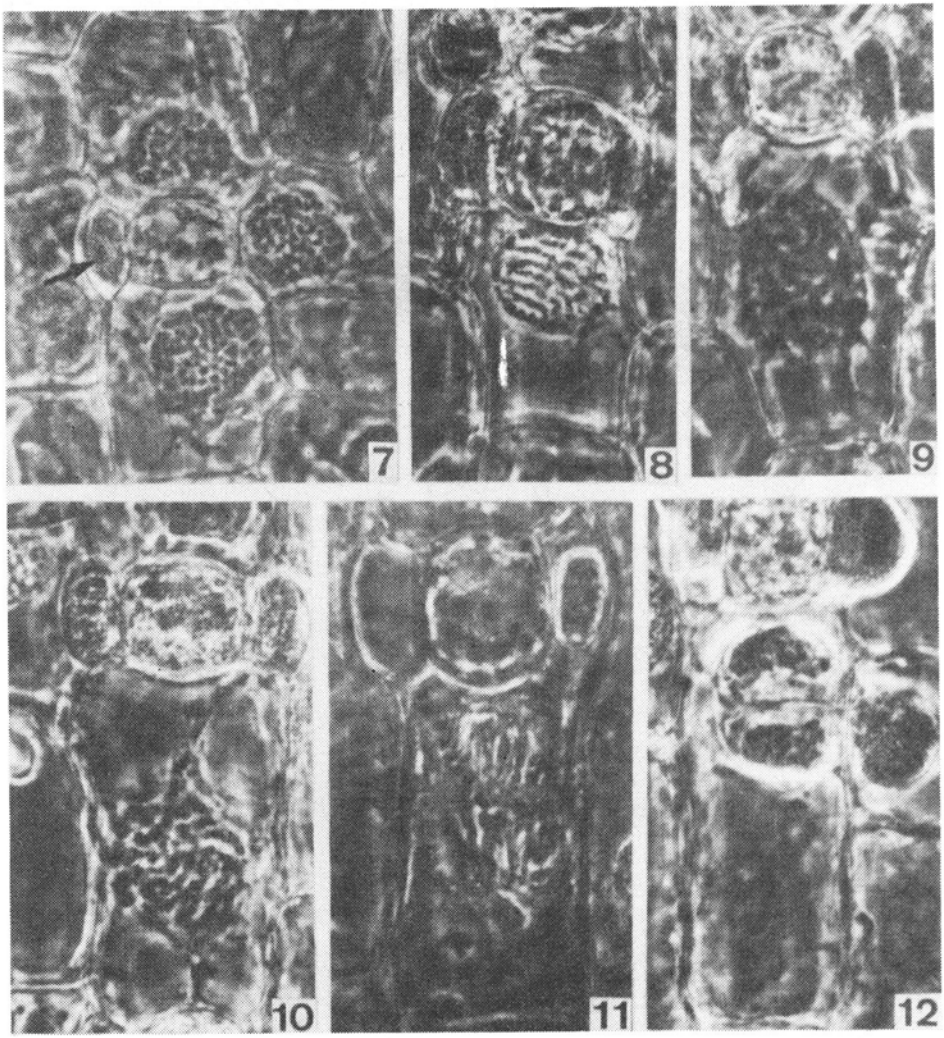


PLATE III

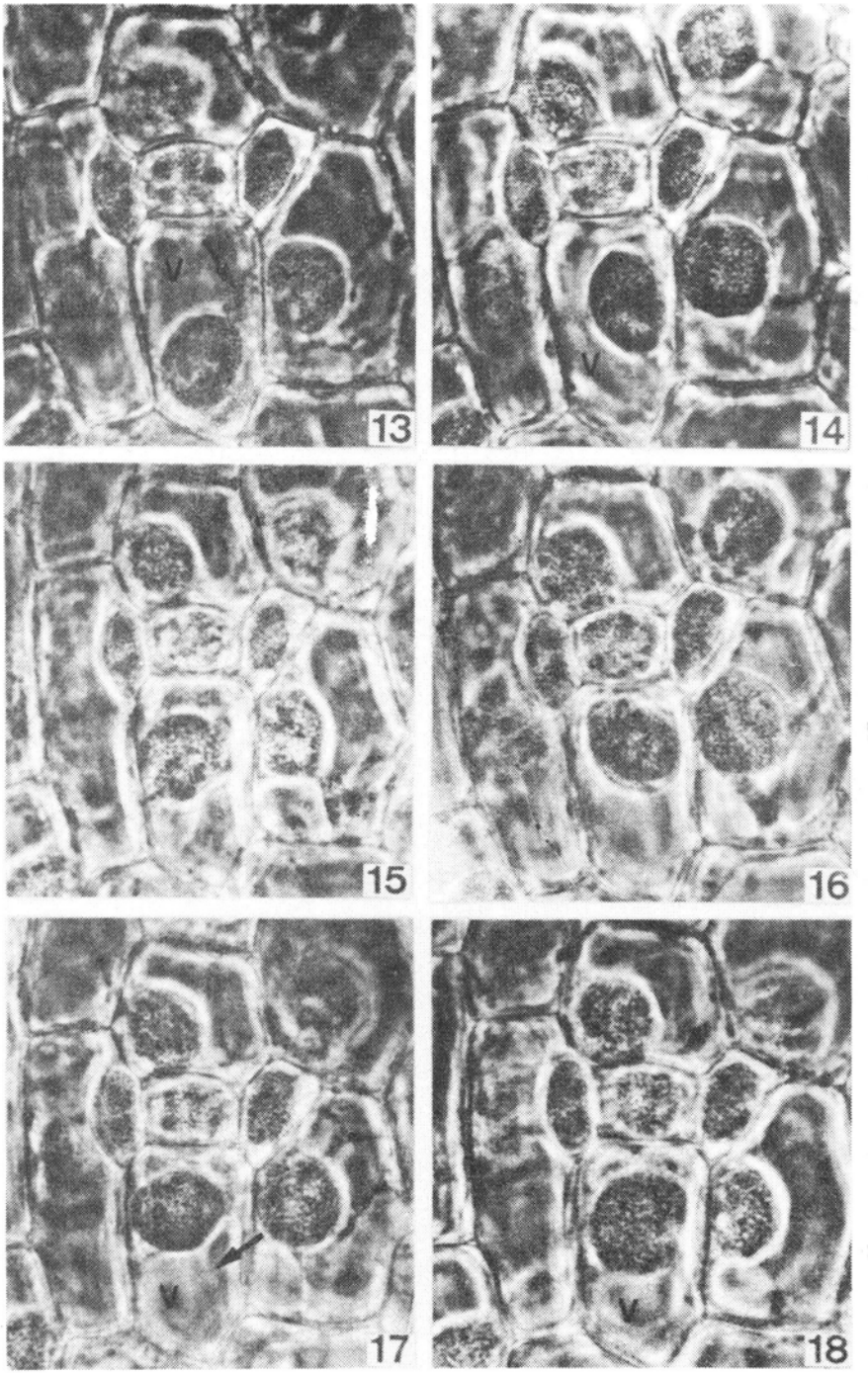
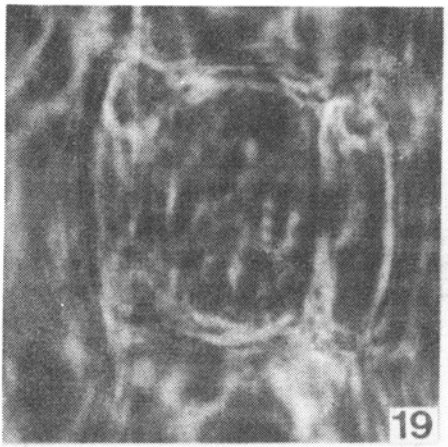
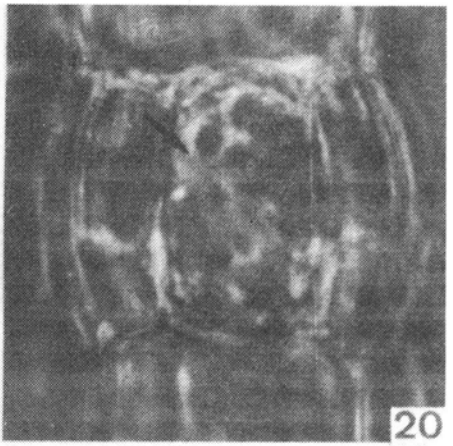
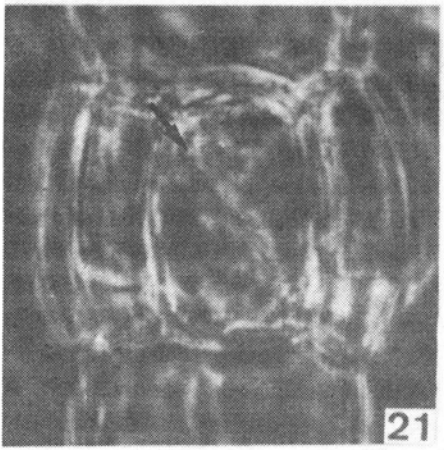
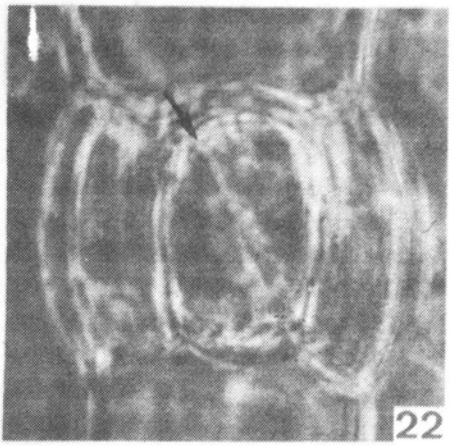


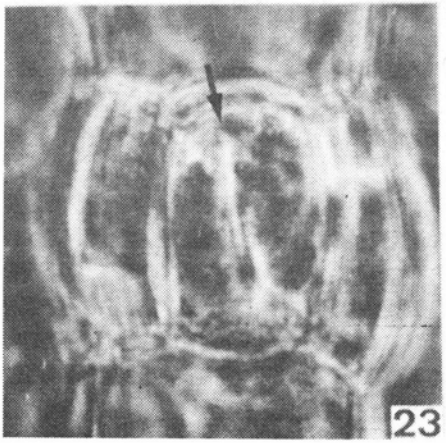
PLATE IV

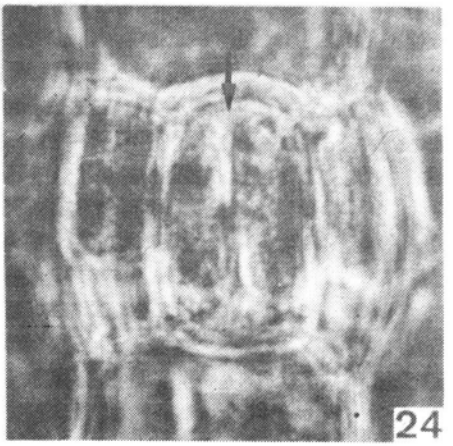












to kidney-shaped) with very long, club-shaped nuclei and small vacuoles at their polar ends. Chloroplasts with starch grains became visible in the cytoplasm (Fig. 1).

DISCUSSION

The ontogeny of stomata, as traced in this investigation, is in agreement with that described by Fryns-Claessens and Van Cotthem (1973). Most of the stomata were formed as the result of a set sequence of mitoses, and the formation of GCMCs and all of the subsidiary cells were usually the result of asymmetric divisions of epidermal cells. These asymmetric divisions were due to the translocation of nuclei and, as shown in this study, the translocations were usually along long and winding paths to the place where the division was to take place. We sought, then, the answer to the question, what causes these translocations of the nuclei.

In EM studies on the ontogeny of stomata in wheat, Pickett-Heaps and Northcote (1966) suggest that it is the preprophase band (PPB) made up of microtubules (MTs) that determines the position of the future cell plate. But the question arises, what causes the specific orientation of the MTs band.

Lindenmayer (1984) discusses various mechanisms determining the position of the PPB and various factors which may be instrumental in setting the plane of cell division. The author emphasizes that these factors are probably different for different tissue types. All the more so, since in the hyphae of fungi, PPBs have not been found. Kaufman et al. (1970) (studies on the ontogeny of stomata in oats) point to the probable, important role of a hormonal factor, both in the translocation of nuclei and in the positioning of the MTs band. This could be oxogenous IAA or GA_3, or yet another hormone.

It can be concluded from the in vitro observations presented in this paper that the movements of the nuclei are linked with changes in the vacuolization of the cell. The size of the vacuoles changes in specific areas of the cell with simultaneous changes in the length of cytoplasmic bridges. Large vacuoles can arise through the joining of smaller ones. The significant role played by vacuoles in the polarization of cells has been established and described many times in embrological material. A polarized, differentiating division, leading to the formation of a generative cell is preceded by extensive vacuolization of the microspore. This has been demonstrated by, among others, Charzyńska (1973), who also explains the translocation of the generative cell from a parietal position to the interior of the vegetative cell by the joint action of the vacuole systems of the cells.

In a study on the polarity of cells during the differentiation of various

types of embryo sacs, Willemse (1981) considers vacuolization as one of the significant factors. The position of vacuoles is probably responsible for the position of the nucleus. However, in spite of ascribing significant importance in the process of differentiation to the phenomenon of changes in vacuolization of the cell, the causes of this phenomenon still remain unknown. Polarized divisions are most surely expressions of morphogenetic regularities, and their direct causes should be looked for there.

The second fact resulting from this investigation is the diagonal division of the GCMC and the following reorientation of the entire mitotic apparatus, which leads to the proper, parellel to the long axis of the leaf, orientation of the two quard cells.

This very interesting phenomenon was analysed in in vitro and EM studies by Palevitz and Hepler (1974a) during the division of GCMCs in the epidermis of *Allium cepa* cotyledons. Palevitz (1986) also analysed in detail the changes in the plane of division (also in *Allium cepa* cotyledons) using video time-lapse. In both papers the authors found that the metaphase plate can be situated at a greater or lesser angle (usually obliquely), sometimes at an angle of 90° to the final position of the newly formed cell plate, that is, along the long axis of the cotyledon. The reorientation of the entire mitotic apparatus takes place during the period of late anaphase--telophase. This takes on average 15-20 minutes in the onion. The question was posed, why does the mitotic apparatus initially form obliquely in the GCMC and what factors or structures move it into the proper, final position?

It can be assumed that the oblique position of the mitotic apparatus (tropometaphase) is the result of the limited internal space of the cell — the spindle takes up the maximum possible space. This was already shown by Tischler in 1921, and confirmed by Bělăr in 1929 (cit. Palevitz and Hepler 1974a). Palevitz and Hepler (1974a) and the authors of this paper agree with this.

The answer to the question on what is the mechanism of reorientation of the mitotic apparatus is difficult. In studies using many approaches including respiration inhibitors, selected antimitotics, and factors inhibiting the formation of the cell plate, Palevitz and Hepler (1974b) showed that a very significant role is played by the connection of the MTs of the edges of the phragmoplast (on both ends of the forming cell plate) with the cytoplasmic MTs grouped by the plasma membrane. But, as is supposed by the authors, in the reorientation of the entire mitotic apparatus, the most important role is played by the constant presence of the microtubular structure of the spindle-phragmoplast. This hypothesis was confirmed by Palevitz (1986). He found that the reorientation of the cell plate lasts until it reaches a position coinciding with the PPB zone. The role of the PPB is thus very singular. It acts as a perfect rotation mechanism quaranteeing

that the cell plate joins with the cortex/plasma membrane (and later with the cell wall of the mother cell) in the proper position. The new cell wall will run along the long axis of the cotyledon. However, the nature of the phragmoplast-cortex/plasma membrane interactions still remains unknown.

Motions within the cell, and so reorientation of the cell plate too, are undoubtedly linked with expenditure of energy by the cell. What then is the source of this energy and with what structural elements of the cell is it connected? The presence of MTs is associated with, among others, the movement of various cellular structures, mainly the movement of chromosomes during mitosis, and with the motion of flagella and cilia of animal and also plant cells. The existence of cross-bridges between MTs and plastids as well as MTs and cell nuclei has been demonstrated, which suggests that the specific movements of organelles are associated with the presence of microtubules (Gunning and Steer 1975). There is, however, no direct evidence that the MTs are responsible for such translocations. Palevitz (1986) is also unconvinced about the source of energy necessary for the reorientation of the mitotic apparatus. Basing on his own studies and data from literature, that author suggests that the microtubule cross-bridges and actin fibers present in the phragmoplast along with tubulin may have a significance in supplying the energy necessary during the reorientation of the mitotic apparatus. It should be emphasized that there is still no direct evidence that the MTs are responsible for the translocation of organelles. It seems that the MTs show the way and form a scaffold, over which the movement of organelles in the cell takes place.

REFERENCES

Charzyńska M., 1973. Translocation of the generative cell towards the centre of the pollen grain due to changes in the vacuolar system. Acta Soc. Bot. Pol. 42: 295-300.

Fryns-Claessens E., Van Cotthem W., 1973. A new classification of the ontogenetic types of stomata. Bot. Rev. 39: 71-138.

Gunning B. E. S., Steer M. W., 1975. Ultrastructure and the biology of plant cells. Publ. E. Arnold, London.

Kaufman P. B., Petering L. B., Yocum C. S., Baic D., 1970. Ultrastructural studies of stomata development in internodes of *Avena sativa*. Amer. J. Bot. 57: 33-49.

Lindenmayer A., 1984. Models for plant tissue development with cell division orientation regulated by preprophase bands of microtubules. Differentiation 26: 1-10.

Palevitz B. A., 1986. Division plane determination in guard mother cells of *Allium*: video time-lapse analysis of nuclear movements and phragmoplast rotation in the cortex. Develop. Biol. 117: 644-654.

Palevitz B. A., Hepler P. K., 1974a. The control of the plane of division during stomatal differentiation in *Allium*. I. Spindle reorientation. Chromosoma 46: 297-326.

Palevitz B. A., Hepler P. K., 1974b. The control of the plane of division during stomatal differentiation in *Allium*. II. Drug studies. Chromosoma 46: 327-341.

Pickett-Heaps J. D., Northcote D. H., 1966. Cell division in the formation of the stomatal complex of the young leaves of wheat. J. Cell Sci. 1: 121-128.
Willemse M. T. M., 1981. Polarity during megasporogenesis and megagametogenesis. Phytomorphology 31: 124-134.

Przemieszczanie jąder i reorientacja aparatu mitotycznego w ontogenezie aparatów szparkowych Tradescantia virginiana L.

Streszczenie

Badano in vitro, w kontraście fazowym ontogenezę aparatów szparkowych w skórce liści trzykrotki *Tradescantia virginiana* L. Dojrzały aparat szparkowy zbudowany jest z 2 komórek przyszparkowych i 4 komórek dodatkowych (pomocniczych): 2 boczne i 2 biegunowe. Aparaty zorientowane są wzdłuż długiej osi liścia. Stwierdzono, że wszystkie 4 komórki dodatkowe tworzone są w wyniku asymetrycznych mitoz w komórkach skórki otaczających merystemoid (komórka macierzysta komórek przyszparkowych) — GCMC. Przed tym różnicującym podziałem jądro przemieszcza się na terenie komórki, pokonując często krętą, długą drogę by ostatecznie powrócić na pozycję premitotyczną, tj. ku ścianie graniczącej z GCMC (fot. 7, 8). Ruchy jąder związane są ze zmianami wakuolizacji komórki, ale bezpośrednia przyczyna tej polaryzacji i asymetrycznego podziału jest nieznana. Ostatni podział kończący rozwój aparatu szparkowego to mitoza w GCMC. Układ chrmosomów w metafazie (płytka metafazowa) jest wyraźnie skośny (zwykle po przekątnej komórki), ale od późnej anafazy i w telofazie cały aparat mitotyczny wraz z powstającą centryfugalnie przegrodą pierwotną podlega reorientacji (fot. 19-24). Ostatecznie, nowopowstała ściana komórkowa oddzielająca obie komórki przyszparkowe położona jest prawidłowo, tj. wzdłuż długiej osi liścia. Nieznane są mechanizmy reorientacji, ale można przypuszczać, że to mitrotubule "wskazują" kierunek i stanowią "rusztowanie" dla zmieniającego położenie aparatu mitotycznego.