

## Cytokinesis in pollen mother cells I. *Tradescantia virginiana*

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It is known that *Tradescantia* represents a successive type of cytokinesis (Beatty and Beatty 1953; Waterkeyn 1962; Sakai and Shigenaga 1964). The cell plate is formed after the first and second division.

Only Waterkeyn (1962) has investigated the structure of the cell plate in the pollen mother cells (PMC) of *Tradescantia*. He described its structure as a granular composition.

There are no data as yet concerning the chemical nature of the cell plate structures in PMC of *Tradescantia*.

In the present investigations an attempt has been made to compare the cytokinesis in PMC of *Tradescantia* with the cytokinesis in somatic cells in higher plants.

### MATERIAL AND METHODS

The PMC of *Tradescantia virginiana* in the first meiotic division were used as material.

The investigations were made on fresh and fixed anthers. Baker's and Carnoy's fixatives were used. The contents of the anthers were pressed out into suitable dyes and substrates. Paraffin sections were prepared (10  $\mu$  thick). Some of the paraffin sections were stained with iron haematoxylin after Heidenhein. Lipids were detected by staining with Sudan III and IV, Sudan black B — in 70 per cent ethanol and Oil Red O dissolved in isopropanol with a simultaneous control after the extraction by pyridin. The mercuric bromophenol Blue method for proteins was applied (Mazia et al. 1953). Resorcinol Blue solution (Eschrich and Currier 1964) was used for the localization of callose. Pectins were detected using an aqueous alkaline Ruthenium Red solution.

Hydrolases were investigated in material fixed with Baker's (Fo-Ca) with the addition of 7.5 per cent of sucrose at 4°C for 18 h, or in fresh material. For non-specific esterase the  $\alpha$ -naphthyl acetate method with diazonium Fast Blue RR was used. The control was incubated in a substrate solution with the addition of 0.01 M NaF (Pearse, 1961). Acid phosphatase was detected by the azo-dye coupling method, of diazonium Fast Blue RR salt. The control was the same as in the case of non-

-specific esterase (Pearse 1961). The Golgi structures were visualized by the thiamine pyrophosphatase (TPPase) method after Novikoff and Goldfischer (1961). Neutral Red solution for staining *in vivo* and the Da Fano method (Romeis, 1948) for silvering the Golgi structures were also applied.

The succinic dehydrogenase reaction with Nitro BT at pH 7.4 was used on fresh material.

Zeiss (Jena) polarization equipment was used.

## RESULTS

Granular structures of about  $0.2\text{--}0.3\ \mu$  in diameter in the equatorial plane of the phragmoplast of *Tradescantia virginiana* are visible after staining with iron-haematoxylin (fig. 1). Staining with Sudan III and IV, Sudan black B and Oil Red O shows small lipid structures situated between these granules. A positive reaction for lipids is also shown by larger granules of the cell plate, not visible after haematoxylin, and by still larger droplets of about  $0.5\text{--}0.9\ \mu$  in diameter located within the cell plate (fig. 2). At a somewhat later stage of the telophase in place of lipid granules — a rift which is formed centrifugally is observed; yet relatively large lipid droplets of about  $1.5\ \mu$  in diameter may be seen inside the rift (fig. 3).

After pyridin extraction no positive reaction with Sudans and Oil Red O in the plane of the cell plate is observed. Mercuric bromophenol Blue reaction shows the

### Plate I

Telophase in pollen mother cells of *Tradescantia virginiana*.

Fig. 1. — fix. Carnoy, stained with Heidenhain's haematoxylin. Note dark granular structure of the cell plate.

Fig. 2. — fix. Fo-Ca Baker, Sudan III and IV staining. Large lipid granules in the cell plate are visible.

Fig. 3. — fix. Fo-Ca Baker, Sudan III and IV staining. Later stage than fig. 2. Note the rift in place of the cell plate granules and the large single lipid globule.

Fig. 4. fix. Carnoy, mercuric bromophenol Blue method. Positive results in the granules of the cell plate.

Fig. 5. fix. Fo-Ca with addition of 7.5 per cent sucrose. Acid phosphatase, coupling method, Fast Blue RR. Strong activity in granules of the cell plate and cytoplasm.

Fig. 6. — fix. Fo-Ca with addition of 7.5 per cent sucrose. Thiamine pyrophosphatase in the granular structures of the cell plate.

Fig. 7. — Da Fano method. Note Golgi structures in the equatorial region of phragmoplast.

Fig. 8. — mat. unfixed. Succinic dehydrogenase using Nitro BT. Unequal distribution of mitochondria in the equatorial region. Early telophase.

Fig. 9. — mat. unfixed. Later stage than fig. 8. Note mitochondria at both sides of the cell plate.

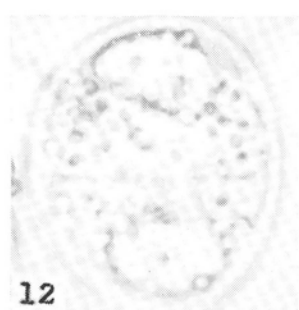
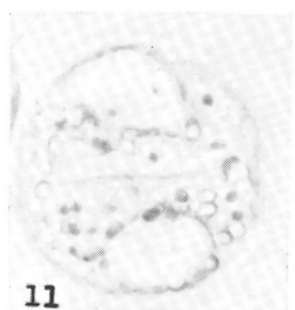
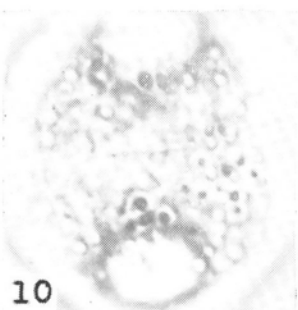
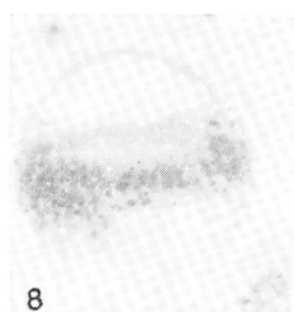
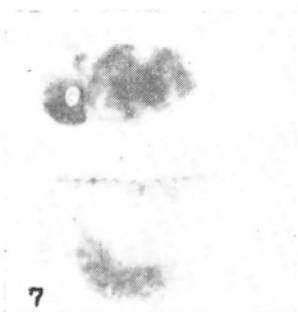
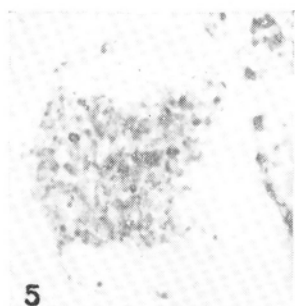
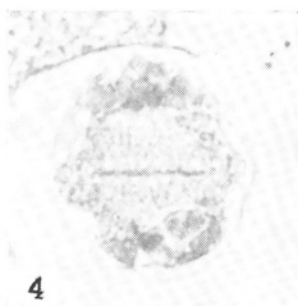
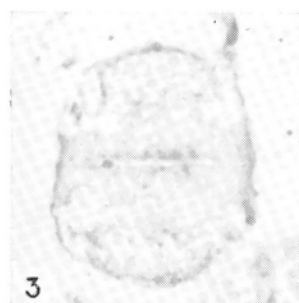
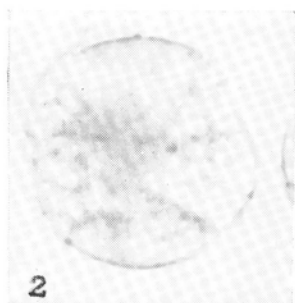
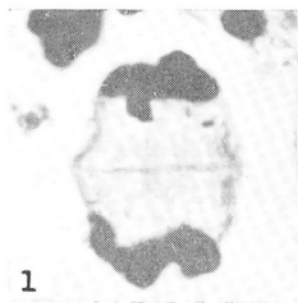
Fig. 10. — mat. unfixed. Neutral Red staining. Note dark structures in the cell plate.

Fig. 11. — mat. unfixed. Stained with Resorcinol Blue. Callose granules are visible in the cell plate.

Fig. 12. — mat. unfixed. Later stage than fig. 11. Note continuous layer of callose at the equatorial region.

Magn. 1000×

Plate I



presence of proteins in the small granular structures of the cell plate (fig. 4). These structures are dark when stained with haematoxylin. An intensive reaction for proteins is also visible in the small granules of the cytoplasm.

The reaction for acid phosphatase reveals its activity in protein granules of the cell plate and in the cytoplasm (fig. 5). A similar localization of the non-specific esterase in the granules can also be seen.

In the division plane of PMC of *Tradescantia* — the Novikoff and Goldfischer's reaction reveals the presence of TPPase (regarded as a marker of Golgi structures) in granular structures of about  $0.5-0.8\ \mu$  in diameter (fig. 6). They are somewhat larger than the protein granules containing acid phosphatase and non-specific esterase. These structures are less numerous in the cell plate than the larger granules which are carriers of hydrolases.

The Da Fano method gives silvering effects in the cell plate of *Tradescantia* — it shows a series of granules of about  $0.5-0.8\ \mu$  in diameter (fig. 7). The Neutral Red in vivo accumulated only a few granules grouped in the cell plate (fig. 10).

In the early telophase, succinic-dehydrogenase was found in numerous mitochondria lying between two daughter nuclei (fig. 8). At the time of formation of the cell plate, the mitochondria stained with formazan are grouped on both sides of it (fig. 9).

It is possible to demonstrate by the Resorcinol Blue staining method that in the division plane of PMC — at a later telophase — blue stained granules appear (fig. 11). They are the beginning of the formation of a continuous callose layer. During the progression of cell division, these granules appear abundantly in the equatorial plane of the phragmoplast and flow together into a continuous callose layer (fig. 12), which is the very beginning of the wall separating the daughter cells. A staining with alkaline Ruthenium Red solution gives negative results in the division plane. Ruthenium Red only stained the wall of the pollen cells — thus confirming the presence of pectins.

Observations of cytokinesis in PMC of *Tradescantia* in polarized light show that only the transversal wall is not birefringent during cytokinesis.

## DISCUSSION

As described above, the cell plate appearing during microsporogenesis of *Tradescantia virginiana* is of a granular character. This complies with Waterkeyns opinion (1962). Investigations of meiosis of *Orchidaceae* with an electron microscope have shown that the cell plate is formed from vesicles and vacuoles (Chardard 1962). In Chardard's opinion, the vacuoles arise by the increase of the volume of vesicles. These vacuoles flow together into a continuous layer thus forming the cell plate.

A quite different opinion is represented by Satô (1959) who described the cell plate in PMC of *Lilium* as a double layer which appears at the beginning of cytokinesis.

An opinion opposite to that of Satô's is presented by Sakai and Shigenaga (1964) in their work on the behaviour of mitochondria in the microsporogenesis of *Tradescantia reflexa*, who only confirmed that mitochondria "are distributed between the daughter cells by the cell plate formed among the mitochondria". In their opinion — mitochondria, lipid granules, short profiles of the endoplasmic reticulum and dictyosomes seem to intrude into the spindle. At the beginning of formation of the cell plate, small vesicles occur not clearly defined by the authors. In fig. 10 of the work these authors show the cell plate with lipid granules; their distribution and dimensions correspond to those of lipid granules described in the present observations.

The cell plate granules in PMC of *Tradescantia virginiana* show the presence of proteins, and some of them not visible after haematoxylin, show the presence of lipids. The protein granules of the cell plate give the reaction for acid phosphatase and non-specific esterase. The chemical character of the cell plate granules suggests a certain similarity with the cell plate granules in somatic cells of *Allium cepa* (Olszewska and Gabara 1964; 1966), in the young petals of *Tradescantia* and in the endosperm of some monocotyledons (Olszewska and Gabara 1964). These authors are of the opinion that the hydrolytic enzymes appearing periodically in the cell plate granules cause a local lysis of the cytoplasm, and, in consequence, the two protoplasts are separated.

The lipid granules in PMC of *Tradescantia* — just as in the onion's meristem (Olszewska 1960) — might appear as a result of dissolving of the lipoprotein complex of the cytoplasm. Granules containing hydrolytic enzymes in PMC of *Tradescantia* also appear abundantly outside the cell plate — in the cytoplasm.

The hydrolase carriers in the onion-scale epidermal cells of *Allium cepa* are spherosomes (Wałek-Czernecka 1962; 1963; 1965). The same conclusion is supported by Górska-Brylass (1965a) with regard to pollen cells and pollen tubes. Matile et al. (1965) have shown biochemically the localization of hydrolases in the spherosomes of tobacco and maize seedlings.

Contrary to the spherosomes described by Wałek-Czernecka and Górska-Brylass, granules with acid phosphatase and non-specific esterase in the cell plate in PMC of *Tradescantia virginiana* do not exhibit lipids.

The presence of thiamine pyrophosphatase has been shown in the cell plate of PMC of *Tradescantia* — in granules as large as spherosome granules or in still larger ones. This enzyme is recognized as a marker of the Golgi apparatus (Novikoff and Goldfischer 1961; Olszewska et al. 1965).

The positive results of Da Fano silvering (Stęplewski and Olkowski 1962) and selective staining with Neutral Red have proved the presence of Golgi structures in the cell plate. These properties are attributed to Golgi structures (Gabara 1965; Olszewska et al. 1965; Palczewska 1966; Gabara, in press).

Numerous investigations with an electron microscope show the part played by Golgi structures in the constitution of the cell wall (Mollenhauer et al. 1961; Whaley et al., 1963; Sievers, 1963; Frey-Wyssling et al. 1964; Bisalputra 1966).

It is also known from cytochemical investigations in a light microscope that Golgi structures appear abundantly in the cell plate in later stages of cytokinesis (Olszewska et al. 1966) and in the zone of the newly-forming transversal wall in the case of *Spirogyra* and *Cladophora* and in the top cells of *Cladophora* (Gabara 1965).

Callose appears already in an early stage of cytokinesis — at first in the shape of granules. Callose granules separate the protoplasts of daughter cells. Weterkeyn (1962) suggests that callose appears during the flow-together time of the granular plate into a continuous layer. However, according to Chardard (1962) — the cell plate has a pectin vacuole character; when the latter flow together, they form a continuous layer of the true cell plate. He distinguishes within the flow-together time a pectin layer which represents the origin of the middle lamella. Yet, Chardard (1962) does not quote any specific method for proving the presence of pectin.

The callose formation mechanism in plant cells still remains obscure. Undoubtedly — by the hypothesis that spherosomes take part in the production of callose (Górska-Brylass, 1965 b). some light is thrown on this problem.

Callose — like other polysaccharides in plant and animal cells (Schnepf 1963; Peterson and Leblond 1964; Dashek and Rosen 1966) — can be secreted by Golgi structures. The convergence between the appearance of Golgi structures and the callose in the division plane of PMC authorized such a conclusion.

### SUMMARY

It has been shown that the cell plate in microsporogenesis of *Tradescantia virginiana* is of a granular character. These granules are characterized by the presence of proteins and by the activity of acid phosphatase and non-specific esterase. Alongside the granules, lipid globules are visible in the cell plate.

Within the time of formation of the cell plate the appearance of Golgi structures (the presence of TPPase, positive results of Da Fano method and selective staining by Neutral Red) is observed.

Callose appears in an early stage of cytokinesis as granules. As cell division progresses these granules flow together into a continuous callose layer.

The author wishes to express her thanks to Docent Maria Joanna Olszewska D. Sc. for very valuable suggestions, kind and constructive criticism and much help during the preparation of this paper. For critical reading of the manuscript, the author is indebted to Docent Bohdan Rodkiewicz D.Sc.

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## REFERENCES

- Beatty I. W., and A. W. Beatty, 1953, Duration of the stages in microspore development and in the first microspore division of *Tradescantia paludosa*. Amer. J. Bot. 40: 593—596.
- Bisalputra T., F. M. Ashton, and T. E. Weier, 1966, Role of dictyosomes in wall formation during cell division of *Chlorella vulgaris*. Amer. J. Bot. 53, 213—216.
- Chardard R., 1962, Recherches sur les cellules mères des microspores des Orchidées. Rev. Cytol. Biol. végét. 24: 1—148.
- Dashek, W. V., and W. G. Rosen, 1966, Electron microscopical localization of chemical components in the growth zone of Lily pollen tubes. Protoplasma. 61: 192—204.
- Eschrich W., and H. B. Currier, 1964, Identification of callose by its diachrome and fluorochrome reactions. Stain. Technology. 39: 303—307.
- Frey-Wyssling A., J. R. López-Saéz, and K. Mühlethaler, 1964, Formation and development of the cell plate. J. Ultrastr. Res. 10: 422—432.
- Gabara B., 1965, Zagadnienie cytokinezy w oparciu o badania nad *Spirogyra* i *Cladophora*. Rozprawa doktorska, Uniwersytet Łódzki.
- Gabara B., 1967, Cytokineza u *Spirogyra* sp. i *Cladophora* sp. Zesz. Nauk. U.Ł. s. II, 23: 113—131.
- Górska-Brylasek A., 1965 a, Hydrolases in pollen grains and pollen tubes. Acta Soc. Bot. Pol. 34: 589—604.
- Górska-Brylasek A., 1965 b, Cytomorfologiczne badania nad kalożą łagiewek pyłkowych. Acta Soc. Bot. Pol. 34: 757—767.
- Matile Ph., J. P. Balz, E. Semadeni, and M. Jost, 1965, Isolation of spherosomes with lysosome characteristics from seedlings. Z. Naturforsch. 20 b; 693—698.
- Mazia D., P. A. Brewer, and H. Albert, 1953, The cytochemical staining and measurement of protein with mercuric bromophenol Blue. Biol. Bull. 194: 57—67.
- Mollenhauer H. H., W. G. Whaley, and J. H. Leech, 1961, A function of the Golgi apparatus in outer rootcap cells. J. Ultrastruct. Res. 5, 193—200.
- Novikoff A., and S. Goldfischer, 1961, Nucleoside diphosphatase activity in the Golgi apparatus and its usefulness for cytological studies. Proc. Natl. Ac. Sci. 47: 802—810.
- Olszewska M. J., 1960, Recherches sur le caractère chimique de la plaque cellulaire. Acta Soc. Bot. Pol. 29: 249—261.
- Olszewska M. J., and B. Gabara, 1964, Recherches cytochimiques sur la présence de certaines hydrolases au cours de la cytotécèse chez les plantes supérieures. Protoplasma. 59: 163—179.
- Olszewska M. J., B. Gabara, M. Kwiatkowska, and I. Palczewska, 1965, Recherches cytochimiques sur la localisation de la thiamine pyrophosphatase dans les cellules végétales. Bull. Soc. Sci. Let. de Łódź. 16,5.
- Olszewska M. J., B. Gabara, and Z. Stęplewski, 1966, Recherches cytochimiques sur la succession d'enzymes hydrolytiques, sur la présence de la thiamine pyrophosphatase et des polysaccharides au cours du développement de la plaque cellulaire. Protoplasma. 61: 60—80.
- Palczewska I., 1966, Über die Lokalisierung der Thiaminpyrophosphatase in den Hyphen von *Achlya* sp. Acta Soc. Bot. Pol. 34: 753—756.
- Pearse A. G. E., 1961, Histochemistry theoretical and applied. J. A. Churchill Ltd., London.
- Peterson M., and C. P. Leblond, 1964, Synthesis of complex carbohydrates in the Golgi region, as shown by radioautoradiography after injection of labelled glucose. J. Cell Biol. 21: 143—148.
- Romeis B., 1948, Taschenbuch der mikroskopischen Technik. R. Oldenburg Verl., München.
- Sakai A., and M. Shigenaga, 1964, The behaviour of mitochondria in pollen mother cells of *Tradescantia reflexa*. Cytologia. 29: 214—225.
- Satō S., 1959, Electron microscope studies on the mitotic figure. II. Phragmoplast and cell plate. Cytologia. 24: 98—106.
- Schnepf E., 1963, Golgi-Apparat und Sekretbildung in den Drüsenzellen der Schleimgänge von *Laminaria hyperborea*. Naturwiss. 50: 674.
- Sievers A., 1963, Beteiligung des Golgi-Apparates bei der Bildung der Zellwand von Wurzelhaaren. Protoplasma. 56: 188—192.

- Stęplewski Z., and Z. Olkowski, 1962; Cytochemical studies on the interrelation between lysosomes and the Golgi zone in uterine epithelium. Bull. Ac. Pol. Sc. seria Sc. Biol. 10, 495—497.
- Wałek-Czernecka A., 1962; Mise en évidence de la phosphatase acide (monophosphoestérase II) dans les sphérosomes des cellules épidermiques des écailles bulbaires d'*Allium cepa*. Acta Soc. Bot. Pol. 31: 539—542.
- Wałek-Czernecka A., 1963; Note sur la détection d'une estérase non spécifique dans les sphérosomes. Acta Soc. Bot. Pol. 32: 405—408.
- Wałek-Czernecka A., 1965, Histochemical demonstration of some hydrolytic enzymes in the spherosomes of plant cells. Acta Soc. Bot. Pol. 34: 573—588.
- Waterkeyn L., 1962, Les parois microsporocytaires de nature callosique. La Cellule. 62: 225—255.
- Whaley W. G., H. Hilton, and H. H. Mollenhauer, 1963, The Golgi apparatus and cell plate formation — a postulate. J. Cell Biol. 17: 216—221.

*Cytokineza w komórkach macierzystych pyłku*  
*I. Tradescantia virginiana*

STRESZCZENIE

Wykazano, że przegroda pierwotna w mikrosporogenezie u *Tradescantia virginiana* ma charakter ziarnisty. Ziarnistości te charakteryzują się obecnością białek i aktywnością kwaśnej fosfatazy i esterazy niespecyficznej. Obok tych ziarnistości w przegrodzie pierwotnej obecne są kule lipidowe.

W okresie tworzenia przegrody pierwotnej pojawiają się struktury Golgiego (Obecność TPPazy, pozytywny wynik metody Da Fano i selektywne barwienie czerwinią obojętną).

Kaloza widoczna jest we wczesnym stadium cytokinezy w postaci ziarnistości, które w miarę trwania procesu podziału zlewają się tworząc ciągłą listwę kalozową.