Electron Microscope Studies on the Mitotic Figure II. Phragmoplast and cell plate¹

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Received December 18, 1958

The fine structure of the metaphase spindle, in his first report of "electron microscope studies on the mitotic figure", has been already described by the present author (1958). In the present paper, both the phragmoplast appearing at the end of anaphase and completed in telophase of plant mitotic cells, and also the cell plate arising in the equatorial zone of the phragmoplast, are by the help of the electron microscope described with reference to their fine structures, and also with reference to the origin and the development of both apparatus. The hypotheses on the formation of the cell wall are also discussed.

Material and methods

The material used was the pollen mother cell of *Lilium lancifolium* Thunb. It was fixed in CdCl_2 solution used by Wada and Fukunaga (1957). This method was already described by the present author in a previous paper (1958). The procedure of preparation of samples was as follows: pollen mother cells, in either anaphase or telophase stage, were fixed in an equivalent mixture of 10^{-1} M CdCl_2 solution and 100 per cent alcohol for 30 minutes. Later, the materials were dehydrated through the alcohol series, and were embedded in 7 parts n-butyl methacrylate and 3 parts methyl methacrylate and polymerized at 45°C . Sections were cut with the Hitachi Ultra-Microtome. The electron microscope used, was the Hitachi Type HU-10.

Results and discussion

The phragmoplast

The fine structure of the metaphase spindle which has been described by the present author in a former paper (1958), is considered to be substantially common to both plant and animal cells. However, the phragmoplast and the cell plate taken up in this paper, can be seen only in the cormophyte mitosis. In the previous investigation (Satô 1958) the pollen mother cell of *Lilium longiflorum* was mainly used, but in this experiment, as the latter

¹ Contributions from the Divisions of Cytology and of Genetics, Botanical Institute, Faculty of Science, University of Tokyo, No. 376.

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out of season, the pollen mother cell of *Lilium lancifolium* was investigated. The pollen mother cell of *L. lancifolium* like that of *L. longiflorum* is also suitable for the research of mitosis by means of the electron microscope. For fixation, CdCl_2 solution by which the fibrous structure of the metaphase spindle has been excellently maintained, was also employed in the present experiments. The fine fibrous structure of the phragmoplast and of cell plate has been disclosed by the electron microscope as in the case of the spindle.

In the space between two separated groups of the daughter chromosomes during anaphase, the phragmoplast which is assumed to be fibrous in structure and barrel-shape in contour, appears in telophase (Fig. 1). From the following characteristics, the phragmoplast is proved to be an independent system originating from the spindle body: 1) its position in the cell is the same space previously occupied by the anaphase spindle, 2) the appearance of the unit fibrils making up the phragmoplast is the same as that of the spindle body, and 3) no cytoplasmic elements are found within it. In Fig. 1, the cell plate appears already in the equatorial zone of the phragmoplast, lying at right angles to the unit fibrils composing the phragmoplast. However, at this very initial stage of the phragmoplast formation, no definite cell plate appears yet.

It has been reported by Inoué (1952) and the others that the phragmoplast *in vivo* shows birefringence under polarized light. Therefore, it is an indisputable fact that the fine structure of the phragmoplast may be composed of anisotropically oriented fibrils at least at submicroscopic level, but electron microscopy concerning the structure of the phragmoplast has hardly demonstrated its fine fibrous structure until now. The phragmoplast as shown in Figs. 1 and 2 is composed of innumerable fine fibrils which are parallel to each other and lie perpendicularly to the equatorial plane, converging toward both poles of the spindle. These fibrils are 250–1000 Å in width, and the thicker ones seem to be built up by aggregation of several fine “unit fibrils”. This order is larger than the unit fibrils in the metaphase spindle which range from 100 to 150 Å, described by the present author in the previous paper (1958). In the phragmoplast can be seen also numerous dense, fine grains having 500–1500 Å in diameter (Figs. 1–3), which are highly possible the same granules scattered in the metaphase spindle.

The fine structure of the phragmoplast and the granules is closely connected with that of the spindle. However, it may not be always conform with the data of the spindle mentioned above, because the material used in this experiment is different from that of the previous experiment, for the material used in the former experiments was *L. lancifolium* and in the latter *L. longiflorum*. It has been demonstrated by the experiments of Wada (1939, 1950) that the phragmoplast substance is induced from the atractoplasm by hydration. Therefore, during the course of the change from atractoplasm into the phragmoplast substance, the swelling of each fibril may be thought

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to occur. Practically the average width of phragmoplast-fibrils is about 600 Å, while that of spindle-fibrils about 120 Å. Moreover, the phragmoplast *in vivo* may contain more fluid as interfibrillar substance than the spindle, in addition to the fibrils and fine granules. The density of this fluid is very low electron-microscopically.

The cell plate

There are no definite explanations on the submicroscopic structure of the cell plate and its developmental process, but this is an important subject which may contain various problems when studied electron-microscopically. So far as I know, the observations of the cell plate by means of the electron microscope have been tried by Rozsa and Wyckoff (1950) and Sedar and Wilson (1951). In the former the root-tip of onion fixed with Flemming's solution, and in the latter the same material fixed with Randolph's chrome-acetic-formalin mixture were used. They observed occasionally the structure and the formation of the cell plate. The formation of the cell plate is one of the most difficult subjects of plant mitosis discussed by many plant cytologists, and it remains unsolved under the usual light microscope.

At the beginning of cell-plate formation, its submicroscopic structure can hardly be detected without help of the electron microscope. The present author has made clear the details of the cell-plate formation at the submicroscopic level by the help of the electron microscope. Fig. 2 shows an early indication of the formation of cell plate, and reveals that the lamellar structure has not yet been formed clearly, but wavy lines across the center portion of the phragmoplast can be seen. They are found under careful observation to be composed of two lamellae, and in each lamella, other unit fibrils are crossing vertically to the unit fibrils of the phragmoplast. When these unit fibrils of a lamella or of young cell plate cross the unit fibrils of the phragmoplast, their points of intersection appear as dark points, or as a line of granules. Therefore, it is not a fact that the fibers of the phragmoplast swell in the equatorial plane and form a line of granules, as was supposed by classic cytologists. Also it can not be recognized that the granular elements in the cytoplasm flow into the phragmoplast and form a line on the equatorial plane, because in Figs. 1-3, there exists a distinct difference of constituents between the phragmoplast and the cytoplasm.

In the beginning of telophase, unit fibrils of the cell plate appear dense in the middle part of the phragmoplast, and light in its peripheral region. These facts may indicate that the unit fibrils of the cell plate are formed at their commencement in the central region of the phragmoplast, and develop step by step centrifugally in one plane toward the periphery of the phragmoplast. Moreover, it is a striking fact that the cell plate appears as a double layer from the beginning of its formation. The unit fibrils of the cell plate accumulate in the equatorial plane as a double layer. The fibrils in either layer which lie intertwined among themselves, increase in number and thus

the space they occupy also increases. Thus two parallel lamellae are formed appearing as one cell plate under light microscope. Then the ground unit fibrils of the phragmoplast between two lamellae disappear gradually (Fig. 3).

The hypotheses concerning the development of the cell plate discussed under the light microscope are then nothing but speculation based on insufficient resolving power of optics as well as on artifacts induced by unsuitable fixatives. Timberlake (1900) has described the formation of the cell plate. He assumed that the fibers of the phragmoplast swell in the equatorial zone, and such swellings fuse with each another in a plane and form a cell plate, and then this new cell plate splits into two layers. Ellenhorn (1933) and Becker (1938) have reported that the initial structure of the cell plate is granular. However their opinions on the origin of granules were varied. Ellenhorn has described that granules flow from the chromosomes, while Becker has considered that granules originate from the cytoplasmic portion. In the electron micrographs obtained by the present author, there exist no such granules in the cell-plate region, as suggested by them. However, the opinion that a series of granules are compressed in the equatorial plane of the phragmoplast and form a cell plate in classic cytology is generally accepted at present. Recently the result obtained by *in vivo* observations of the mitosis has shown that the cell plate appears from the beginning of its development as a line across the center of the phragmoplast (Wada 1950).

Even under the light microscope, if one observes closely, it can be observed that a completed cell-plate consists of two lamellae, and it was generally thought that one lamella is formed at first and then it secondarily splits into two lamellae. The observation by means of the electron microscope has demonstrated that the cell plate is built up of two lamellae from its initiation as mentioned above.

The cell plate shown in Fig. 3 is a completed one in which two parallel lamellae or three layers can be seen if an interval space between two lamellae is added. Among these layers two outer lamellae show high density to the electron beam, and each has 250–1000 Å in thickness, and the interval region is 2500–4000 Å in width.

The cell wall

The next problem is the relationship between the cell plate and the formation of the cell wall. Furthermore, we must resolve the relationship between the cell wall and the development of the plasma membrane. There are some opinions on these points. According to Yamaha (1926) and Becker (1932), the substances contributing to the cell wall formation appear *de novo* between two layers which develop as the cell plate grow up to the plasma membrane of the daughter cells respectively. Another opinion is that the cell-plate substance is transformed into the cell wall. Under the supposition that the cell plate is composed of a lamella, it was interpreted that the cell-wall substances deposit along both sides of the cell plate which

transforms itself into a middle lamella between two daughter cells (Robyns 1924, 1926). Yasui (1939) considers the cell plate as an initial middle lamella and that this initial middle lamella is formed by the compression of many fine granules, and transforms itself further into the middle lamella by addition of callose. However, she has scarcely described the developmental process of the cell wall.

Fig. 4. Part of daughter cells in late telophase showing centripetal growing of the cell-wall substances (ws) diffusing from the mother cell wall along the cell plate.

The results of the observation by means of electron microscope, in Fig. 4 of this paper, show that the cell-wall substances are accumulated centripetally from the mother cell wall into the space between two lamellae consisting of a cell plate. Therefore, it is considered that two lamellae develop into one *de novo* cell membrane between two sister cells. That the hardening of

the cell wall develops centripetally was also confirmed by other experiments (Wada 1939, 1950). He reported that the cell plate transforms centripetally into the daughter cell wall by hardening from the periphery to the middle part of the cell plate. From the data described in the present observations, contributions to the formation of the plasma membranes of the daughter cells, could hardly be obtained.

The present author has chiefly described the submicroscopic structure of the phragmoplast and of the cell plate by means of the electron microscope. At submicroscopic level there exist intimate relationships between cytochemical research and morphogenesis. The unit elements taking part in the cytokinesis should be considered as such macromolecules whose molecular structures should be compatible with the morphological results obtained in the electron micrographs. In future such subjects should be discussed submicroscopically as well as biochemically.

Summary

1. The fine structure of the phragmoplast, that of the cell plate, and its formation in meiosis has been examined by means of the electron microscope in pollen mother cells of *Lilium lancifolium* fixed with CdCl_2 alcohol solution.

2. The barrel-shaped phragmoplast is consisted of innumerable parallel fibrils lying vertically to its equatorial plane, and of the fluid substances with lower density to the electron beam, which fill up the interfibrillar space.

3. The cell plate appears from its initiation as two parallel layers composed of unit fibrils which are different from those of the phragmoplast. The unit fibrils of the cell plate lie crossing vertically crosswise to the unit fibrils of the phragmoplast.

4. The cell plate develops centrifugally in the phragmoplast along its equatorial plane.

5. After the cell plate reaches the mother cell wall, cell-wall forming substances from this mother cell wall are diffused into the space between the two lamellae thus making one cell plate. The hardening of the daughter cell wall develops centripetally.

The author wishes to express his appreciation to Professor B. Wada for his encouragement and suggestion. The author is also indebted to Dr. N. Tanaka and Mr. S. Sakata for criticism and technical help in carrying out this work. This work was supported in part by a grant from the Science Research Fund, Ministry of Education, and in part by a research grant from the Fujii-Kai Research Encouragement Fund.

Literature cited

- Becker, W. A. 1932. Experimentelle Untersuchungen über die Vitalfärbung sich teilender Zellen. Studien über Zytokinese. Acta. Soc. Bot. 9: 381-409.

- 1938. Recent investigations *in vivo* on the division of plant cells. Bot. Rev. 4: 446-472.
- Ellenhorn, J. 1933. Experimental-photographische Studien der lebenden Zelle. Zeits. Zellf. und Mikr. Anatomie 20: 288-308.
- Inoué, S. 1952. The effect of colchicine on the microscopic and submicroscopic structure of the mitotic spindle. Exp. Cell Res. Suppl. 2: 305-318.
- Robyns, W. 1924. Le fuseau du caryocinèse et le fuseau cytotinèse dans les divisions somatiques des Phanerogames (I. part). La Cellule 34: 367-454.
- 1926. Ditto (II. et III. part). La Cellule 37: 145-177.
- Rozsa, G. and Wyckoff, R. W. G. 1950. The electron microscopy of dividing cell. Bioch. Biophys. Acta 6: 334-339.
- Satô, S. 1958. Electron microscope studies on the mitotic figure. I. Fine structure of the metaphase spindle. Cytologia 23: 383-394.
- Sedar, A. W. and Wilson, D. F. 1951. Electron microscope studies on the normal and colchicized mitotic figures of the onion root tip (*Allium cepa*). Biol. Bull. 100: 107-115.
- Timberlake, H. G. 1900. The development and function of the cell plate in higher plants. Bot. Gaz. 30: 73-99, 154-170.
- Wada, B. 1939. Experimentelle Untersuchungen lebenden Zellen in der Teilung. IV. Die Einwirkung des Dampfgemisches von Ammonia und Chloroform auf die Mitose bei den *Tradescantia*-Haarzellen. Cytologia 10: 158-179.
- 1950. The mechanism of mitosis based on studies of the submicroscopic structure and of the living state of *Tradescantia* cell. Cytologia 16: 1-26.
- and Fukunaga, K. 1957. A new technique to demonstrate the surface membrane of the spindle in preparations. Cytologia 22: 442-451.
- Yamaha, G. 1926. Über die Zytokinese bei den Pollentetradenbildung, zugleich weitere Beiträge zur Kenntnis über die Zytokinese in Pflanzenreich. Jap. Jour. Bot. 3: 139-162.
- Yasui, K. 1939. On the cytokinesis in some angiosperms, with special reference to the middle lamella initial (MLI) formation and the phragmoplast. Cytologia 9: 557-574.

Explanation of plates VI-VII

Plate VI

Fig. 1. An electron micrograph of a longitudinal section of a pollen mother cell of II telophase in *Lilium lancifolium* showing the fine fibrillar structure of the phragmoplast and those of the young cell plate crossing the former. The young cell plate appears partly as double parallel lines. CdCl_2 fixative.

Plate VII

Fig. 2. A phragmoplast containing the very young cell plate. Neither an accumulation of granules nor knobbed fibrils appear in the equatorial region of phragmoplast.

Fig. 3. A longitudinal section of the phragmoplast in which the cell plate appears as two parallel wavy lines crossing perpendicularly to each unit fibril of phragmoplast.