Behavior of Membrane System in the Cell During Cell Divisions of Microsporogenesis in *Tradescantia paludosa*

I. Premeiotic Mitosis

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Various changes of membrane system during somatic mitosis in plants have been studied with an electron microscope by PORTER and MACHADO (1960) in root cells of *Allium cepa* and *A. sativum*. They found that the nuclear envelope breaks down into pieces of cisternae which become indistinguishable with the cytoplasmic elements of the endoplasmic reticulum. These cisternae reconstitute the new nuclear envelope, and are involved in formation of the cell plate.

During formation of a pollen grain, there are different types of successive cell divisions; premeiotic, meiotic and postmeiotic mitoses. The aim of the series of studies is to compare these different types of cell divisions at the submicroscopic level, with special reference to the behavior of the membrane system of the cell.

Present paper concerns with the premeiotic mitoses of sporogenous cells in young anthers of *Tradescantia paludosa*.

Materials and Methods

Young anthers of *Tradescantia paludosa* were fixed in 2% aqueous solution of potassium permanganate for two hours at room temperature. They were, then, washed in 30% ethanol and were dehydrated through a series of ethanol. Materials were embedded in an Epon mixture (LUFT, 1961). The resin mixture used is composed of Epon 812, dodecynil succinic anhydride (DDSA) and methyl nadic anhydride (MNA) with a ratio of 7/6/4, and contains 1.5% of DMP-30 as an accerelator. Thin sections were cut by an ultramicrotome and were examined with an electron microscope.

Observations

Sporogenous cells undergoing premeiotic mitoses are packed together closely, and show different stages of the mitoses even in one cross section (cf. Fig. 1).

In the lower right cell in figure 1, the nucleus contains electron dense, anastomosing threads of 0.3 to 0.5 μ in width and a round body of about 2.5 μ in diameter. When thick sections are stained by Feulgen staining or with Azure B, the anastomosing threads are Feulgen positive, and are identified as chromonemata or chromatin threads. The round body is Feulgen negative and is stained violet with Azure B, and may be the nucleolus. As the width of the chromonemata in this cell is the thinnest in this tissue, the cell seems to be at interphase or very early prophase. The nucleus is surrounded by a continuous nuclear envelope of about 300 A in thickness. In the cytoplasm there are several long slender profiles of cisternae which are distributed more or less evenly among such cell-organelles as plastids. The thickness of the cisternae is similar to that of the nuclear envelope and measures 300 A. Both the nuclear envelope and the cisternae have slightly electron dense contents when observed at an higher magnification.

The lower left cell in figure 1 is probably at mid-prophase because the chromonemata are thicker than at earlier stage. The nucleus in this figure contains two nucleoli. There is still a continuous nuclear envelope, which shows prominent waving at this stage. Those points of the envelope which contact with the chromonema concave into the nucleus. No change is noticeable in number of the cisternae from the earlier stage which is seen in lower right cell in figure 1.

The cell shown in figure 2 is assumed to be in late prophase because neither the continuous nuclear envelope nor the nucleolus is seen. There is a larger number of cisternae in this cell than in the early prophase cells in which the continuous nuclear envelopes are recognizable. It should also be noted that the cisternae seen in this figure are often longer than those at previous stages. These facts seem to suggest that the nuclear envelope breaks down into somewhat long pieces of cisternae, but not into small vesicles.

In the cell at upper right in figure 1, chromosomes are arranged on the equatorial plane. The arrow indicates the spindle axis. In this metaphase cell, numerous cisternae are concentrated in polar regions. These cisternae are more or less parallel to the spindle axis. Most of the plastids and the mitochondria surround the concentrated cisternae at the polar regions.

Figure 3 shows an anaphase cell, in which chromosome separation has started. The cisternae, the plastids and the mitochondria are still in the polar regions. As indicated by an arrow at the upper pole in this figure, the cisternae run more or less in parallel with each other and curve to embrace the spindle at the pole. Such figures are obtained only when the direction of the section is proper, and show longitudinal profiles of a cup- or umbrella-like structure.

When the chromosomes have arrived at the mitotic poles (Fig. 4), most of the cisternae, the plastids, the mitochondria and the Golgi bodies are seen between the two groups of daughter chromosomes. Most of the cisternae are in the central

portion of the cell, and are more or less parallel to the mitotic axis. It should be noted that parts of some cisternae contact with the distal parts of the chromosomes, as indicated by arrows.

Figure 5 seems to show a late anaphase or early telophase cell, in which chromosomes are found at mitotic poles, but the individual chromosome is indistinguishable. Mitochondria and plastids are disposed to present in the periphery of the cell, while the cisternae are either on the surface of the chromosome mass or in the central portion of the cell. The equatorial side of the chromosome mass is surrounded by long, continuous cisternae as indicated by an arrow, whereas the polar side of the chromosome mass is surrounded by discontinuous cisternae. This fact suggests that many cisternae unite to form a continuous nuclear envelope, and that the reconstruction of the envelope starts from the equatorial side of the daughter nuclei. It should be emphasized here that some short cisternae remain on the equatorial plane.

At telophase a continuous nuclear envelope is completed around the two daughter nuclei. In addition to the short cisternae, there are numerous vesicles in the equatorial zone (Fig. 6). When observed at an higher magnification (Fig. 7), two kinds of vesicles are distinguished; smaller and larger. The size of the "small" and "large vesicles" are about 300 to 500 A and 700 to 1000 A in diameter, respectively. Both kinds of vesicles are enclosed by a single membrane of about 80 A in thickness. The "large vesicles" have electron-less-dense contents, whereas the "small vesicles" contain slightly electron-dense materials. The electron density of the contents in the "small vesicles" and in the cisternae is similar. Sometimes the "small vesicles" are found near the end of the cisternae. These facts suggest that the "small vesicles" are formed from the cisternae by a process of pinching off.

In figure 8, a layer of about 700 A in width is seen on the equator between the daughter nuclei. The layer is composed of an electron-less-dense space and two electron-dense, parallel membranes of about 80 A in thickness. At the end of the layer there are the "large vesicles". These facts suggest that the layer is an initial cell wall, the membrane being the plasma membrane, and that the initial cell wall is probably formed by fusion of the "large vesicles". The "small vesicles" are fewer in this cell than in a cell at the previous stage. A few cisternae are still present near the newly formed cell wall. In the following stage, all the cisternae soon scatter more or less evenly in the cytoplasm.

Discussion

Behavior of the membrane system in the cell during premeiotic cell divisions in the sporogenous tissue is more complex than that of other cell organelles such as plastids and mitochondria, that is, the cell divisions accompany break-down and reformation of the nuclear envelope and formation of the new plasma membrane. In respect to the way of the nuclear envelope break-down and to the distribution and movement of the cisternae, premeiotic mitosis in *Tradescantia paludosa* is essentially similar to such somatic one as in root cells of *Allium* reported by PORTER and MACHADO (1960).

Present observation indicates that the nuclear envelope breaks down into somewhat long cisternae, which mix with the cisternae in the cytoplasm. These cisternae participate in formation of the new nuclear envelope and plasma membrane at anaphase and telophase.

When the nuclear envelope breaks down, it has been reported that intensive vesiculation of the envelope occured (Moses, 1958; Merriam, 1959; Barer et al., 1959). However, this phenomenon was not observed in the material used in this investigation. Whereas there is only small number of cisternae in the cytoplasm at interphase and prophase of sporogenous cells, there are numerous cisternae in the cells in which the nuclear envelopes have broken down. The increase in number of the cisternae at pro-metaphase seems to have to do with the break-down of the nuclear envelope. It is highly probable to conclude that the nuclear envelope breaks into pieces of cisternae, which are, then, released into the cytoplasm and mix with the cisternae which have originally been in the cytoplasm.

At metaphase the cisternae concentrate into two groups in the polar regions of the dividing cell. As the chromosomes separate towards each pole, the cisternae migrate to the equatorial zone. These cisternae differentiate into three classes of membranes in the cell; the nuclear envelope, the plasma membrane and the cisternae in the cytoplasm.

The process of reconstruction of the nuclear envelope is as follows. In the early stage of this process a part of a cisternae adheres to a distal portion of an anaphase chromosome. In the next stage when the chromosomes aggregate into a mass, many cisternae surround the chromosome mass and fuse with each other to form a complete nuclear envelope. It seems to be unlikely that parts of the original nuclear envelope have an individuality and reorganize the new nuclear envelope. The result obtained in this study agrees with the finding of Amano and Tanaka (1957) who have observed that the nuclear envelope develops by fusion of fragments of endoplasmic reticulum.

The other cisternae, which are not used for the construction of the nuclear envelope, stay on the equatorial zone and participate in formation of cell wall and plasma membrane. Light microscopic observations showed that pectin vesicles appear on the equator and fuse to form a continuous new cell wall (Becker, 1936). By use of an electron microscope Porter and Machado (1960) have observed that the cisternae form a rather tight latticework on the equator, and that the pectin vesicles appear within the lattice. They suggested that small, restricted loci in the spindle matrix were lysed or otherwise changed and were enclosed by a membrane. Mazia (1961) speculated that the collection and fusion of the cisternae presents an ideal solution to the problem of formation of new plasma membrane between the daughter cells. In the present study it was found that the "small vesicles" appear first on the equator. They contain materials which are as electron dense as the cisternae. They are often found near the ends of the cisternae. These facts suggest that the "small vesicles" are pinched off from the cisternae. The electron-less-dense "large vesicles" appear later and increase in number. They fuse with each other and form a new cell wall. Although the "large vesicles" are of submicroscopic order, it would

seem that they correspond to those pectin vesicles of BECKER (1938) which were found by light microscopic observations. The succession and the mode of appearance of the cisternae, the "small vesicles" and the "large vesicles" on the equator at telophase suggest that the cisternae pinch off the "small vesicles", which transform to the "large vesicles" by accumulation of some wall substances. Thus, the plasma membrane which borders the cell wall may originate from the membranes of the cisternae. Recently WHALEY and MOLLENHAUER (1963) have reported that the vesicles produced from the Golgi bodies arrange on the equatorial plane and form the cell wall. In the sporogenous cells in *Tradescantia paludosa,* only a few Golgi bodies are seen, and they do not seem to be responsible for the formation of the new cell wall.

Several cisternae remain near the newly formed cell wall. They soon scatter more or less evenly in the cytoplasm.

Summary

Premeiotic mitosis in *Tradescantia paludosa* was observed with an electron microscope. The nuclear envelope shows waving and, then, disintegrates into pieces of cisternae, which mix with those which have been in the cytoplasm. These cisternae migrate to the polar regions of the cell and show a cup- or umbrella-like arrangement. At late anaphase the cisternae move to the interchromosomal region. Some of them, then, attach to the chromosomes and reconstruct a new nuclear envelope. The other cisternae vesiculate on the equatorial zone, and participate in formation of the new plasma membrane and the cell wall. Rest of the cisternae becomes scattered more or less evenly in the cytoplasm.

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Legend of Plates

Plate I

Figure 1.   Group of cells undergoing premeiotic mitosis in a young anther of *Tradescantia paludosa*.   There are interphase (lower right), prophase (lower left) and metaphase (upper right) cells.   c : cisternae in the cytoplasm.   Ch : chromosomes. nl : nucleolus.   Arrows in the prophase cell show places of the nuclear envelope which concave into the nucleus.   A line in the metaphase cell indicates the direction of the spindle axis.   ×4.000.

Plate II

Figure 2.   A late prophase cell, in which the nuclear envelope has broken down.   The cisternae (c) are concentrated in a restricted area of the cell.   × 4,500.

Figure 3.   An anaphase cell.   The cisternae (c) are concentrated at polar regions of the spindle.   A line indicates the direction of the spindle axis.   Ch : chromosome. ×4,500.

Figure 4.   An anaphase cell in which chromosomes have arrived at the mitotic poles. Cisternae (c) are present in the interchromosomal region.   Arrows indicate the cisternae which contact with the chromosomes.   G. : Golgi body.   ×3,000.

Figure 5.   A late anaphase cell.   Arrows show cisternae which lie on the chromosome mass.   There are also short cisternae (c) in the interchromosomal region. ×4,500.

Plate III

Figure 6.   A telophase cell.   Many vesicles (v) arrange on the equator.   c : cisternae. N : nucleus.   ×11,000.

Figure 7.   A portion of the plate in a late telophase cell.   There are "small vesicles" (sv) which contain slightly electron dense contents.   They are often at the tips of the cisternae as indicated by an arrow.   There are also a few "large vesicles" (lv) which have electron-less-dense contents.   ×36,000.

Figure 8.   A late telophase cell.   "Large vesicles" (lv) are at the end of the continuous layer of the initial cell wall (CW).   c : cisternae.   N : nucleus.   ×15,000.

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