

丸山圭藏

主 論 文

Behavior of Membrane System in the Cell
during Microsporogenesis

(花粉形成時における細胞内膜系の行動)

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Cyclic Changes of the Golgi Body during Microsporogenesis
in Tradescantia Paludosa

Short Title: Cyclic Changes of the Golgi Body

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Despite of extensive studies of the Golgi bodies in animal cells with electron microscopes for the past 10 years, any developmental change of the organelle from smaller structure has not been known. The changes in structure of the Golgi body hitherto reported are a hypertrophy of its cisternae and a pinching-off of vesicles. These are understood to be related to the function of this organelle in accumulating or condensating substances (cf. Dalton, 1961) and may be regarded to be rather degenerative than developmental. Therefore, it is generally believed that the Golgi bodies multiply by division of the pre-existent ones (De Robertis et al., 1960).

On the other hand, some structures composed of concentrically arranged cisternae have appeared in recent published electron micrographs mostly of plant cells, in which they were denoted as Golgi bodies or as dictyosomes (eq. Whaley et al., 1960, Porter, 1961). Recently in the previous preliminary report (Maruyama et al., 1962) we could throw light upon the significance of the ring-structure in terms of the Golgi body development, and showed that the ring-structure is no other than the young form of this organelle. In pollen grains of Tradescantia paludosa at the stages from postmeiotic mitosis to shortly after

pollen maturity a single ring-shaped cisterna grows to a multi-layered ring-structure composed of many concentric cisternae, which then transform itself into a familiar form of the Golgi body composed of a stack of straight and parallel cisternae. In the present study we further examined the developmental change of the Golgi body in earlier stages of pollen development and found that there occur cycles of synchronous development of the Golgi bodies followed by their simultaneous degeneration during these stages. In the following we shall present the cyclic changes of the Golgi body seen during the formation and maturation of the pollen grains in T. paludosa, including these in the stages observed in the previous study.

Materials and Methods

Anthers of Tradescantia paludosa were cut into two halves. One half of the anthers was used as the material for electron microscopic observation. In the cases of the anthers containing pollen mother cells, these half anthers were fixed in toto in 2 per cent potassium permanganate for a time period ranging from 10 minutes to two hours. In the cases of the anthers containing pollen grains, the

pollen grains were pressed onto agar-gel plates and were fixed either in 2 per cent potassium permanganate for two hours or in 5 per cent potassium permanganate for 2 to 5 minutes. For identification of the stages of pollen development, another half of the anthers was smear-stained with aceto-carmin and was examined with a light microscope.

Besides the pollen mother cells and the pollen grains, stigma cells and root tip cells were also used as the material. In the former cases, tips of styles of T. paludosa were fixed in 2, 3, 5, or 5 per cent solution of potassium permanganate for one and a half minutes or for two minutes, and in the latter cases, root tips of T. paludosa were fixed in 2 per cent potassium permanganate for two hours.

Fixation was carried in either case at room temperature. After fixation, the materials were dehydrated in a series of ethanol, and were embedded through propylene oxide in an Epon mixture composed of Epon 812, DDSA and MNA (Luft, 1961) in a ratio of 7 : 6 : 4 (Maruyama, 1963). Thin sections were picked up on naked grids and were examined in an electron microscope without any further treatment.

Observation

At leptotene of the meiotic prophase, there are, in the cytoplasm of pollen mother cells, no typical Golgi bodies which are composed of stacks of more or less straight and parallel cisternae. However, in addition to such cell-organelles as mitochondria, the plastids and cisternae of the endoplasmic reticulum, there are ring-structures of about 0.4 to 0.7 μ in diameter, which are composed of several concentric layers (Figs. 1, 2 and 3). Number of these ring-structures are usually three to five in each cell. There is some variation in number of layers making up of the ring-structure to a certain extent from one cell to another; namely, the ring-structures may be composed of two to three layers in one cell, while four to five in another. In the ring-structure of small size, which is composed of two to three layers, all the layers make a concentric ring (Fig. 1), while in the ring-structure of large size, which is composed of more than three layers, the outer layers do not form complete rings (Figs. 2 and 3). The innermost ring in the ring-structure measures about 0.3 to 0.4 μ in diameter. The distance from one layer to adjacent one is about 300 A. Each layer is resolved to be triple; two electron dense lines

of about 80 A and the middle lighter space of about 100 A. Serial observations suggest that the electron-dense lines are sheet-like and may represent a view of cross section of membranes. When the outer layers do not form complete rings, two paired membranes fuse at both ends. These facts suggest that the layer is a flat sac or a cisterna, which is bent to take a ring form. Length of the cisternae in the ring-structure corresponds, if they are stretched out, to that of the cisternae in the typical Golgi body observed in the present study and are usually 1 to 1.5 μ . Excepting that cisternae are not arranged in straight, the ring-structures are, therefore, essentially similar in organization to the typical Golgi body hitherto described in literatures (cf. Whaley et al., 1959 a; Voeller, 1964).

In the next stage (pachytene) of the pollen mother cell development, in addition to those cells which still contain the ring-structures composed of four to five cisternae, there appears cells which have the typical Golgi bodies and the intermediate form between the ring-structure and the typical Golgi body. The intermediate form is such a structure as composed of still strongly curved cisternae which, however, do not form complete rings any longer as shown in figures 4 and 5. The typical Golgi bodies are

similar to those which have hitherto described as the Golgi bodies in plant cells and basically homologous with the animal Golgi bodies (cf. Dalton and Felix, 1956). They are composed of stacks of more or less straight and parallel cisternae as seen in figures 5 and 6. The distance between two cisternae, the width of the membranes and the space of the cisternae are similar to those in the ring-structure, and measure about 300 A, 80 A and 100 A, respectively. The length of the cisternae is 1 to 1.5 μ . The number of the cisternae in one Golgi body varies from four to six. Sometimes all the pachytene cells in one anther locus contained only typical Golgi bodies, and no ring-structures were found at all. The organization as well as the successive appearance of the ring-structure, the intermediate form and the typical Golgi body during the course of the pollen mother cell development lead to surmise that the ring-structure is no other than the young form of the Golgi body, which develops to the typical body by open-out of its ring-cisternae.

At diakinesis, metaphase and later on, it was difficult to find the Golgi body in pollen mother cells. Figure 7 shows a Golgi body at diakinesis which is composed of a stack of a few cisternae with which small vesicles are associated at the tips. It would seem that the Golgi bodies

are now degenerating by pinching-off of vesicles from the cisternae. Indeed, we could not find out any form of the Golgi bodies after metaphase of the first division of meiosis till the end of meiosis. During this period, even cisternae of the endoplasmic reticulum were seldom seen in the cytoplasm. Figure 8 shows a part of the cytoplasm of microspore tetrads, in which there are but small vesicles ranging from 300 A to 1000 A in diameter and small vacuoles as membraneous elements in the cytoplasm. The small vesicles are preferentially present in groups.

When each microspore separates from the tetrad, rings composed of two concentric membranes appear mostly among the clusters of the vesicles (Figs. 9, 10 and 11). The diameter of the rings is from 0.3 to 0.4 μ , which is approximately the diameter of the innermost ring in multilayered ring-structures found in the pollen mother cell. The distance between the two membranes is approximately 100 A, although it varies to 200 A. Sometimes the outer membrane protrudes to form an extending tail. Such a figure suggests that this ring is a single ring-cisterna.

Later when the cell-vacuoles in the microspore become larger by fusion and swelling, there are multilayered concentric ring-structures composed of two to four cisternae

in the cytoplasm (Figs. 12 and 13). The size of these ring-structure measures 0.5 to 0.7 μ in diameter. The size of the ring-structure and the number of the cisternae increase as development of the microspore proceeds, as judged by the growing size of the vacuoles in the microspore. The width of the cisternae in these ring-structures is more or less constant and measures 100 A. The innermost ring has a similar size as the single rings in the previous stage, and is 0.3 to 0.4 μ in diameter. It seems, therefore, probable that the multilayered ring-structures develop from the single ring-cisterna by addition of other concentric cisternae.

When a huge cell-vacuole has formed in the microspore, the cisternae of the ring-structure show prominent swelling (Figs. 14 and 15). This hypertrophy of the cisternae occurs at one side of the structure and results in an opening-out of all the cisternae (Fig. 16). The structure with the open cisternae are composed of four to five cisternae which measure about 1 to 1.5 μ in length and are associated with small vesicles at the tips of the cisternae. Such structures are, no doubt, of the Golgi bodies similar to those found in various kinds of plant tissues and species. The above mentioned sequence of the morphological changes suggests that the multilayered ring-structures grow from the single rings

composed of one cisterna, and in turn transform themselves into the familiar structure of the Golgi body by opening-out of the ring-cisternae.

During the postmeiotic mitosis, the Golgi bodies further degenerate, and alternate again with new young Golgi bodies. Both the degenerating Golgi bodies having dilated cisternae with associated vesicles and the ring-structures composed of one to four cisternae are seen in the microspores in the same anther loculus, not merely because the anther used in this study contained various stages of mitosis, but also because the development of the Golgi bodies does not correlate exactly with mitotic cycle. One may see the degenerating Golgi bodies in the cytoplasm at anaphase stage, while the ring-structures at prophase stage. Occasionally, there are microspores which contain both the degenerating Golgi bodies and the ring-structures as shown in figure 17. The earliest occurrence of the ring-structure could be traced back to prophase stage, and the latest occurrence of the degenerating Golgi bodies up to telophase stage. However, after the completion of mitosis, there are neither mature Golgi bodies nor degenerating one.

During the maturation of the pollen grains after the postmeiotic mitosis, the development of the Golgi bodies

could be most clearly followed in the cytoplasm around the vegetative nucleus (Maruyama et al., 1962). Immediately after the mitosis, Golgi bodies are of ring form composed of one to four cisternae (Fig. 18). Later when the generative nucleus has elongated into an ellipsoid in shape, the ring-structures become composed of more cisternae, from five to 10 at the maximum and the size of the structure measures 0.7 to 0.8 μ in diameter. Even in the large ring-structure the length of the cisternae does not exceed more than 1.5 μ because the outer layers do not form complete ring (Fig. 18). When the generative nucleus has elongated further into a crescent in shape, there are no ring-structures at all. Instead, there are structures, composing of strongly curved cisternae. In these cases, none of the curved cisternae forms a complete ring (Fig. 19). There are also the typical Golgi bodies at this stage of pollen maturation. Later when the petals of the flower-buds have turned purple in color, all the Golgi bodies in the pollen grain are composed of more or less straight and parallel cisternae of 1 to 1.5 μ in length, the number of which ranges from six to ten (Fig. 21). When the flower has opened, the Golgi bodies in the pollen grain are composed of decreased number of cisternae, namely four to six, which show prominent hypertrophy at the tips and are

often associated with small vesicles (Fig. 22). In the cytoplasm around the generative nucleus, there are also a few ring-structures composed of one to four cisternae immediately after the mitosis (Fig. 18). Later as the pollen grains mature, it becomes, however, difficult to find the Golgi bodies because the cell elongates very much and the cytoplasm becomes a thin layer lining around the nucleus. Nevertheless, one can see occasionally in the mature pollen grain the Golgi bodies composed of four to five, more or less straight cisternae, when the petals of the flower-buds are purple in color as shown in figure 20. Such a Golgi body has not only small number of cisternae but also has very short cisternae, which measure about 0.5 μ in length, whereas those in the Golgi bodies in the cytoplasm around the vegetative nucleus measure from 1 μ to 1.5 μ .

As mentioned above, the development of the Golgi bodies during the period from the leptotene of meiosis to the maturation of the pollen grains takes place more or less synchronously in all the cells. Now we should like to go back to the earlier stages of microsporogenesis, namely, pre-meiotic mitosis and pre-leptotene stage of meiosis, for the purpose to see if such a synchronous development of the

Golgi bodies takes place there, too. During the premeiotic mitosis, we found both ring-structures and typical Golgi bodies in the same cell (Fig. 23), and it was not possible to find any correlation between the forms of the Golgi bodies and the stages of mitosis. At the end of the premeiotic mitosis, the sporogenous cells enter interphase one by one. Thus, getting to keep step with each other, all the cells in one anther loculus advance into meiosis simultaneously. At the so called preleptotene of meiosis, we found the Golgi bodies only rarely in the cytoplasm. All the Golgi bodies we could find were either degenerating form of the Golgi bodies with a few cisternae in the pollen mother cells found in one anther (Fig. 24) or small ring-structures composed of two to three cisternae in those found in another anther (Fig. 25). Thus, it seems that though the development of the Golgi bodies is not synchronous during postmeiotic mitosis it gets synchronized at the preleptotene stage by degeneration of all the pre-existing Golgi bodies and by development of new young Golgi bodies.

We have further observed some somatic tissues such as root meristems, elongating zone of the roots, and stigmas. One can see ring-structures (Fig. 27) as well as the typical Golgi bodies (Fig. 28) in root meristematic cells, occasionally

even both in the same cell (Fig. 26). It should be noted here that rings of about 0.5 μ diameter are found besides the Golgi bodies. The rings are not made of layered structure, but are composed of one electron-dense ring of about 0.1 μ thick and a central lucent core of about 0.3 μ diameter. This structure is, thus, apparently different from the ring-structure of the Golgi bodies in organization. Such a structure was not found in other tissue cells examined. Similarly, in the cells of the elongating zone of the roots, both the degenerating and the ring forms of the Golgi bodies are seen in the thin layer of the cytoplasm (Fig. 29). In the stigma cells, too, there are various types of the Golgi bodies in the same cell (Fig. 30). Therefore, it seems to be rather general for various forms of the Golgi bodies to co-exist in the same cell of somatic tissues.

Discussion

Although the function of the Golgi body has been inferred to be secretion of substances as this organelle develops highly in sensory cells (cf. Dalton, 1961), it was not until when the phase-contrast and ^{the} electron microscopy were introduced that the true existence of the Golgi body

itself in situ has been established (Dalton and Felix, 1953 and 1956; Sjöstrand and Hanzon, 1954; Afzelius, 1956; and others). Especially in plant cells, the existence of the Golgi body had been strongly denied before 1957, when structures which are homologous to the animal Golgi body were reported in various plant tissues and species by electron microscopy (Potter, 1957; Heitz, 1957 a, b and c; Buvat, 1957 a and b; Buvat and Carasso, 1957; Perner, 1957; Sager and Palade, 1957; Lance, 1957). It is now well established that the Golgi bodies have essentially similar basic organization both in plant and animal cells, and are composed of stacks of more or less straight and parallel cisternae with often associated vesicles or vacuoles. In animal cells several of these Golgi bodies are often collected into a group, and may further unite with each other to form a large, the so called Golgi field at certain site in the cell and thus become visible with an ordinary light microscope. On the contrary, the Golgi bodies in higher plants are more or less evenly distributed and present singularly in the cell. Thus the investigation of the Golgi bodies in plants was postponed until electron microscopes became available in cytological laboratories.

Despite of extensive observations with electron micro-

scopes in many laboratories for the past 10 years, no such remarkable metamorphosis of the Golgi body as to indicate that the Golgi body develops through some morphological changes from the younger form was found (cf. de Robertis et al., 1960), except for grouping and unification of the organelles in animal cells and for hypertrophy and pinching-off of vesicles from the cisternae, nor was it possible to obtain an evidence which suggests that the Golgi body grows from some smaller structure composed of fewer cisternae (cf. Whaley et al., 1959 a). Therefore, it is generally believed that the Golgi body multiplies by division of the pre-existent one. Actually there are reports that presented electron micrographs of Golgi bodies possibly undergoing division (Grasse, 1957; Buvat, 1958; Gatenby, 1960; Gatenby and Tahmisian, 1960; Dalton, 1961).

On the other hand, when we survey old literatures, we find a peculiar morphoplasm in plant cells which was insisted as the plant Golgi body. Bowen (1927) found osmiophilic ring formations or "platelets" in kidney beans and spermatozoids of moses after prolonged osmium impregnation, and homologized them to the Golgi bodies in animal cells based mainly on their osmiophilic property (1928 a and b). Such osmiophilic platelets were also observed by Gatenby and

his associates (1928). Beams and King (1935 and 1939) and Jones (1938) found further, after centrifugation of plant cells, that the osmiophilic platelets have specific gravity which differs from that of cellular components such as mitochondria, plastids and lipid granules, supporting the view of Bowen at least to the extent that the osmiophilic platelets are discrete structures in plant cells. However, Bowen's view met strong opposition (Guillermond, 1930 and 1941; Kiyohara, 1930; Newcomer, 1946) and the platelets were thought as an artefact produced by capricious treatment of the cells by osmium impregnation and were taken as vacuome or derivatives of broken plastids or mitochondria. Weier (1931) also did not think that they were the Golgi bodies, though he believed the presence of it as a distinct cellular body in situ. Press (1957), who examined the platelets with the electron microscope, concluded that they were not the Golgi bodies because of dissimilarities of their structure from that of the Golgi body. However, one can find, from some recently published electron micrographs, the ring-structures composed of concentrically arranged circular cisternae (eg. Porter, 1961; Whaley et al., 1960), which remind us of the osmiophilic platelets of Bowen. Heitz (1957 c) first noted, by use of an electron microscope,

the occurrence of the ring-structures which were different from the endoplasmic reticulum or from the typical Golgi bodies known at that time. Later, rings composed of concentric cisternae appeared in many published papers, in which they were denoted as the Golgi bodies or dictyosomes without any reference of the osmiophilic platelets of Bowen, nor without any analysis of the structure. But, it seems, in fact, reasonable to identify them as the Golgi bodies, because the size and the organization of the ring-structure are essentially similar to those of the typical Golgi bodies excepting that the cisternae are strongly curved to form rings, and furthermore because there are structures in which some cisternae are ring while others are more or less straight. These structures do not seem to be an artefact, for they are seen in the materials fixed with various fixatives, such as osmium (Heitz, 1957 c), chrom-osmium (Schneff, 1961), and permanganate for different fixation periods from one and a half minutes up to two hours (Porter, 1961; Whaley et al., 1960; and others).

Schneff (1961) tried to correlate the ring-structure with regeneration of the Golgi body. He thought that the old degenerating Golgi bodies with a few cisternae renew in such a manner that each of their cisternae curves to

form a ring which splits to form two straight cisternae, and thereby the Golgi body regains increased number of cisternae. Mercer (1962) observed that phospholipids in ovotestis of snails form concentrically arranged lamellae which, then, open out to form parallel and straight lamellae. He applied this scheme to the case of the Golgi body, cisternae of which are deduced to be composed of phospholipids and proteins like other membranes in the cell. He postulated that the Golgi body develops from the ring-structure to the familiar structure of the Golgi body composed of a stack of more or less straight and parallel cisternae by opening-out of the ring-cisternae. Our preliminary report (Maruyama et al., 1962) has actually demonstrated that the Golgi body does develop from a ring-structure to the familiar structure known as the Golgi body during the development of pollen grains in T. paludosa.

The present observation confirms the previous finding and extends it further. Namely, both in the pollen mother cells and in the microspores after meiosis as well as in the pollen grains after the postmeiotic mitosis, the ring-structures appear always in younger stages of cell development, and subsequently, there occurs typical form of the Golgi bodies composed of stacks of straight cisternae. Finally, the

Golgi bodies become composed of decreased numbers of cisternae which are swollen and associated with small vesicles at the tips. These facts lead us to a conclusion that the Golgi bodies develop from a ring-structure to a stack of straight and parallel cisternae, and further degenerate by pinching-off of vesicles from the cisternae, periodically three times during the formation and maturation of pollen grains. This way of development of the Golgi body affords us a possible explanation for the shape of the mature Golgi body. Usually, the cisternae of the Golgi body are bowed slightly if not forming rings. Such a structural polarization of the Golgi body seems to be a relic of the young form of the organelle. The cisternae in the middle of the typical Golgi body are often longer than those at both sides. This may result from the fact that in the multi-layered ring-structure the inner ring-cisternae have small diameter and the outer cisternae do not form a complete ring. By the latter fact, the length of the cisternae does not exceed more than 1.5 μ even in the large ring-structure composed of many cisternae. In the typical Golgi bodies the length of the cisternae is 1.5 μ at the maximum. Therefore, there seems no change in length of the cisternae after opening-out of the ring-cisternae until they vesiculate later. Usually the cisternae

in the middle of the typical Golgi body are longer than those at both sides. This may result from the fact that in the multi-layered ring-structure the inner ring-cisternae have small diameter and the outer cisternae do not form complete ring. It should be noted that the development of the Golgi body is rather linear and we could not find any evidence that the old degenerating Golgi body renews by forming ring-cisternae as was thought by Schneff (1961). Furthermore, one ring-cisterna seems to form one straight cisterna by opening-out, for there is no remarkable increase in number of the cisternae which constitute a single Golgi body after opening-out. Although we have no explanation at present how the number of the cisternae increases, the growth of the Golgi body takes place most prominently when the body is in a ring form. Starting from a single ring, the ring-structure grows larger by addition of the cisternae and becomes composed of 10 cisternae at the maximum as seen in the pollen grains after postmeiotic mitosis. This growth of the ring-structure provides an evidence that the Golgi body does develop from a smaller structure to larger one. Thanks to their ring form, young Golgi bodies are easily distinguished from the old degenerating form of the Golgi bodies which are also composed of fewer cisternae.

If the ring-structure seen in many electron micrographs and the Bowen's osmiophilic platelets be identical, this identification of the ring-structure as the young form of the Golgi body implicates that ring form of the osmiophilic platelets of Bowen might represent the young Golgi bodies. However, there are some ring-like formation of unknown nature in root meristematic cells, which could be also the osmiophilic platelets. It seems to be likely that most, if not all, of the platelets may correspond to the ring-structure.

Summarizing the developmental pattern of the Golgi body we can distinguish three stages; growing, mature and degenerating. In the first stage, Golgi bodies are of ring-structure which enlarges by addition of concentric cisternae. In the second stage, the cisternae in the organelle are opened out and are more or less straight and parallel. In the third stage, the organelle shows prominent swelling and vesiculation of the cisternae. These Golgi bodies are composed of decreased number of cisternae, suggesting that the degeneration of the organelle is brought about by pinching-off of vesicles from the cisternae. Although we could not find any connection of the Golgi-cisternae with the endoplasmic reticulum in the present observation,

substances may be transmitted by some way to the Golgi body from the endoplasmic reticulum (Essner and Novikoff, 1962; Zeigel and Dalton, 1962; Kajikawa, 1963). It has been demonstrated that these vesicles which are pinched off from the Golgi-cisternae contain the substances accumulated by this organelle (Mollenhauer et al., 1961; Sano, 1962; Whaley and Mollenhauer, 1963; Mollenhauer and Whaley, 1963). The production of these vesicles is, therefore, the manifestation of active function of this organelle in condensation and secretion of the substances, as was already insisted by light microscopic observations. In this sense, this stage may also be considered as functional stage. Unlike plastids and mitochondria, the Golgi body consumes the structure itself by pinching-off the vesicles from the cisternae when it shows an active function. Such three stages of the Golgi body development are most typically seen in the pollen grains after the microspore mitosis. Namely, synchronization of the development is most strict during this period, the ring-structure grows very large and becomes composed of ten cisternae at the maximum, and there is quite a definite mature stage when all the Golgi bodies are composed of straight cisternae which do not show swelling at all. In the microspore, however, there is no clear mature stage,

which merges in the degenerating stage, for the ring-cisternae in the growing stage show prominent hypertrophy and pinching-off of the vesicles. In the pollen mother cell, there is only small number of the Golgi bodies in one cell, and the developmental stage of the organelle differs from one cell to another (Fig. 31).

Besides that the ring-structures are present in the materials fixed with different fixatives such as osmium, osmium-chrom and permanganate as refered above, the ring-structures appear together with the typical Golgi bodies in the same cell in somatic tissues such as roots and stigmas. These facts again suggest that the ring-structure is not an artificial formation produced only in certain stages of the pollen development in T. paludosa in particular, and lead further to surmise that the Golgi bodies in these somatic tissues may develop similarly from the ring-structure. Since the ring-cisterna can be seen even in a Golgi body of mammalian tissue (see figures 7 and 8 b of Mollenhauer and Zebrun, 1960), this way of development might be applicable even to the animal Golgi body. It seems likely that the somatic cells contain Golgi bodies at different developmental stages, and that if we could observe individual Golgi body with the lapse of time, the organelle might reveal a

similar course of development even in these somatic cells. At any rate, it is rather a striking fact that the development of the Golgi bodies in the pollen is synchronous with the differentiation of the cell, which, in turn, proceeds synchronously in one anther locus. Our unpublished observation suggests that the development of mitochondria and plastids proceeds also synchronously during the pollen differentiation. It seems to be an interesting fact that there occurs such a synchronized development of the cell-organelles as the basis of the synchronous differentiation of cell population. The synchronization of development of the Golgi bodies begins with that of the cell when meiosis commences. During the premeiotic mitosis both the development of the Golgi bodies and mitotic cycle of the cell are not synchronous. After successive divisions of premeiotic mitosis, cells enter into interphase one by one, and then all the cell in one anther locus start to undergo meiosis simultaneously. At this critical stage, we saw the old degenerating form of the Golgi bodies in the pollen mother cells found in one anther, and the young small ring-structure in the pollen mother cells found in another anther. Although we could not know which one of the two anthers contains the pollen mother cells at advanced stage, it seems

to be reasonable to deduce that all the Golgi bodies which existed during the premeiotic period degenerate, and the new Golgi bodies start to develop at this stage. This synchronous development of the Golgi bodies also raises a problem about the correlation between cell metabolism and function of the Golgi bodies. Their only function we know is the condensation and secretion of some substances, and on the other hand, the hypertrophy and the vesiculation of their cisternae occur only once during the formation of pollen grains. When all the Golgi bodies degenerate during the formation and maturation of pollen grains, the post-meiotic and meiotic cell divisions and the elongation of pollen tube take place which will require an elaborated amount of cell wall substances. However, we could not get any positive evidence to show that the Golgi bodies participate in the formation of cell wall, as was proposed by Whaley and his co-workers (1960 and 1963).

The synchronous development of the Golgi bodies is followed by a simultaneous degeneration of the organelle. It is, therefore, clearly recognized that there are, during the formation and maturation of pollen grains, three generations of the Golgi bodies which match with the development of the cells; the first generation of

the Golgi bodies is found in the pollen mother cells, the second in the microspore after meiosis and the third in postmeiotic mitosis. Although we could not find the Golgi body at metaphase of the first division of meiosis, Shigenaga (personal communication) observed ring-structures in metaphase cells. Whether these ring-structures develop to form another generation of the Golgi bodies or not needs further examination. At any rate, the change in generation of the Golgi bodies takes place during cell divisions; namely during the second division of meiosis and during postmeiotic mitosis. During the postmeiotic mitosis sometimes old and new generations of the organelles are occasionally seen concomitantly in one cell. During meiosis, however, there is a period when no Golgi bodies are seen; namely, from meiotic second division to microspore tetrad stage. This period is, therefore, suitable to study the origin of the Golgi body in pollen mother cells and microspores. Single rings, the youngest form of the Golgi bodies, are observed among the clusters of small vesicles in the cytoplasm of the young microspores, suggesting their possible origin from these vesicles. Further study is under way on the nature of these vesicles.

Summary

The development of the Golgi body during the microsporogenesis of Tradescantia paludosa was observed by use of an electron microscope. It was found that all the Golgi bodies develop more or less synchronously in the pollen mother cell, in the microspore after meiosis, and in the pollen grain after postmeiotic mitosis. The youngest form of this organelle is a single ring-cisterna. The organelle enlarges by addition of other concentric cisternae. These arrays open out to form a stack of parallel and straight cisternae, which later decreases in number by vesiculation.

As the synchronous development of the Golgi bodies is followed by simultaneous degeneration, three generations of the Golgi bodies are recognizable during the formation and development of the pollen grains; the first generation is found in the pollen mother cell, the second in the young microspore, and the third in the pollen grain after the postmeiotic mitosis. The change in generation of the organelle takes place during mitoses, namely during meiotic mitosis and postmeiotic mitosis.

In somatic cells such as root meristems, elongating zone of roots, and stigmas as well as in the sporogeneous cells in young anthers, there are various forms of the Golgi bodies in the same cell, indicating that the Golgi bodies do not develop synchronously. Their synchronous development during the formation of pollen grains is, thus, a striking incident, which starts from preleptotene of meiosis.

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Explanation of Figures

Figures 1 - 7. Development of Golgi bodies in pollen mother cells. 1. Ring structure composed of three cisternae. Leptotene stage. X 30,000. 2 and 3. Ring structures composed of 4 - 5 cisternae, of which outer ones do not form complete rings. Leptotene. X 19,000. 4. Golgi body composed of strongly curved cisternae, any of which does not form a complete ring. Pachytene. X 37,000. 5. Golgi body at the right is composed of somewhat curved cisternae, whereas that at the left is composed of straight and parallel cisternae. Pachytene. X 28,000. 6. Mature form of Golgi body composed of more or less straight and parallel cisternae. The uppermost cisternae in the figure shows hypertrophy at their tips. Pachytene. X 37,000. 7. Degenerating Golgi body composed of decreased number of cisternae which are associated with small vesicles. Diakinesis. X 33,000.

Figure 8. A portion of cytoplasm of a microspore tetrad. Any form of Golgi body can not be found, nor cisternae are present in the cytoplasm. Instead, there are groups of small vesicles in the cytoplasm (arrows). N; nucleus, V; vacuole, CW; cell wall. X 37,000.

Figures 9 - 11. Rings composed of one cisterna in young microspores which are just separated from tetrads after meiosis. 9. Arrows indicate tails extending from the rings. X 25,000. 10. Two rings are associated to form dumbbell-shape. X 34,000. 11. Rings and vesicles in the cytoplasm between the nucleus (N) and a vacuole (V). X 21,000.

Figures 12 - 14. Multi-layered ring-structures in microspores which contain a few, large vacuoles. 12. Ring-structure composed of two concentric ring-cisternae. X 34,000. 13. Ring-structure composed of three cisternae, of which outermost one does not form a ring and is swollen at one extremity. PW: pollen wall. X 28,000. 14. Ring-structures, the cisternae of which show a prominent swelling. X 24,000.

Figures 15 and 16. Maturation of Golgi body in microspores, in which one large vacuole has developed. 15. The uppermost organelle in the figure is still ring in form, while in the lower organelle all the cisternae are opened. Both show prominent swelling of the cisternae. X 24,000. 16. Golgi bodies showing extensive vesiculation. Vesicles line up at the tips of the cisternae. N: nucleus. X 25,000.

Figure 17. Golgi bodies at late prophase of postmeiotic mitosis. There are ring (r) and degenerating (d) forms of the Golgi body in one microspore. V; vacuole. X 20,000.

Figures 18 and 19. Golgi bodies in young pollen grains after postmeiotic mitosis. 18. Immediately after the mitosis, when the generative nucleus is round in shape. All the Golgi bodies in vegetative (marked r) and generative cytoplasms (Indicated by an arrow) are of ring form. VN; vegetative nucleus, GN; generative nucleus, W: cell wall between generative and vegetative nuclei. X 22,000. 19. Later stage when the generative nucleus has elongated to ellipsoid in shape. Golgi bodies take either large ring form or intermediate form in which cisternae are almost opened. They are composed of seven to eight cisternae. X 54,000.

Figures 20, 21 and 22. Golgi bodies in mature pollen grains. 20. When petals of flower-buds are purple in color. Golgi bodies are all matured and are composed of seven to eight straight cisterna in vegetative cytoplasm. Arrow indicates a Golgi body in the generative

cytoplasm. GN: generative nucleus, W: cell wall.
X 20,000. 21. Higher magnification of a Golgi body of
mature form. X 45,000. 22. When the flower has
opened. A Golgi body of degenerating form composed of
four straight cisternae. Arrows show swelling of the
cisternae. X 50,000.

Figure 23. Golgi bodies in a cell undergoing premeiotic
mitoses. There are ring (r), mature (m) and degenerating
(d) forms of the Golgi body in one cell. X 20,000.

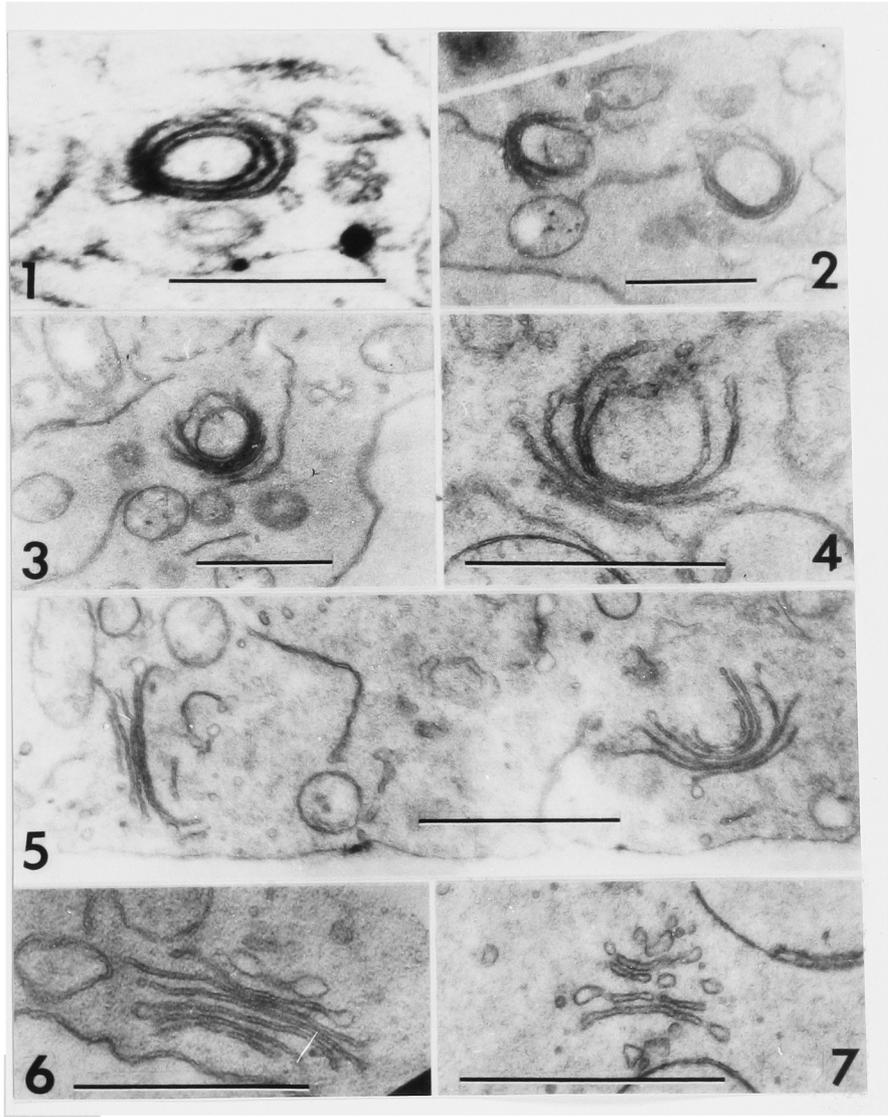
Figures 24 and 25. Golgi bodies at preleptotene of
meiosis in the pollen mother cells found in two different
anthers. 24. Degenerating Golgi bodies showing vesiculation
of the cisternae. X 20,000. 25. Ring-structures (arrows).
X 19,000.

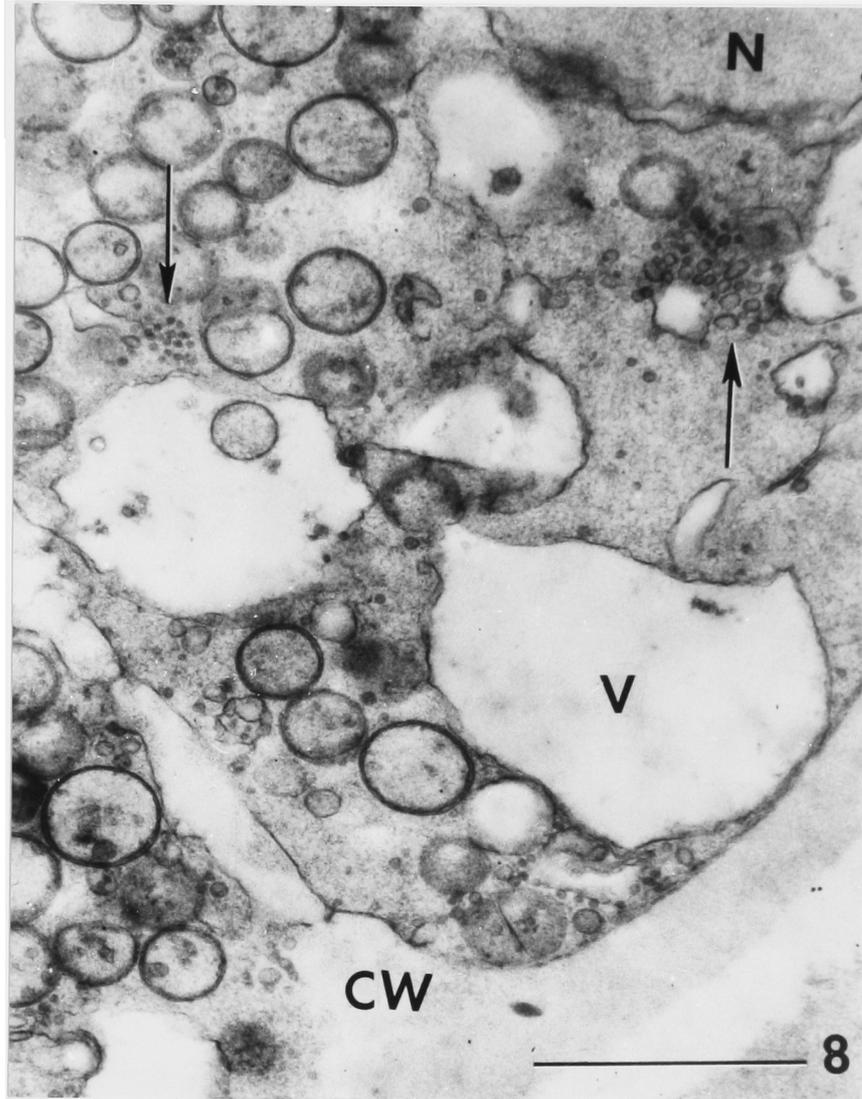
Figures 26, 27 and 28. Golgi bodies in root meristematic
cells. 26. Ring (r), mature (m) and degenerating (d)
forms of the Golgi body in one cell. X 20,000.
27. Ring form of the Golgi body (r) and electron dense
rings of unknown nature (arrows). X 20,000.
28. Mature form of the Golgi body (m) and rings of un-
known nature (arrows). X 20,000.

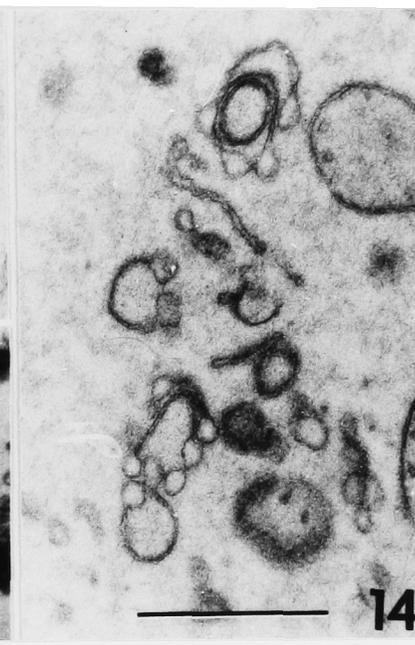
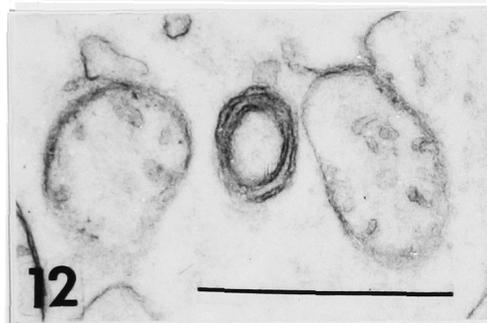
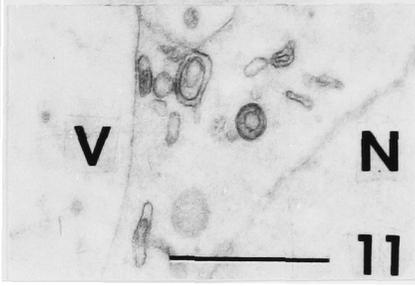
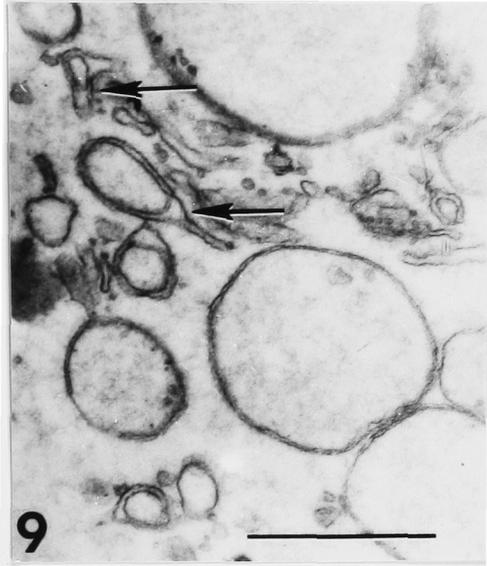
Figure 29. Golgi bodies in a differentiated cell in elongating zone of root. There are ring (r) and mature (m) forms of the Golgi body in a thin layer of cytoplasm between the cell wall (W) and a huge vacuole (V). X 20,000.

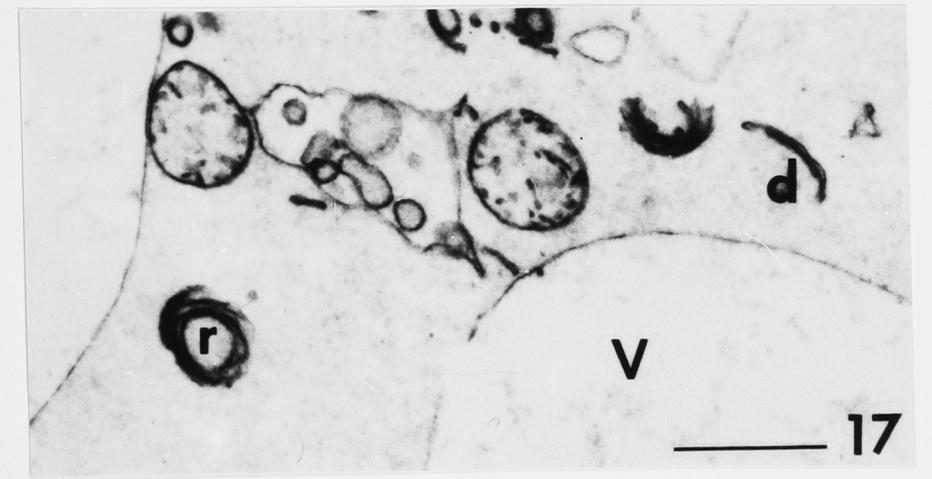
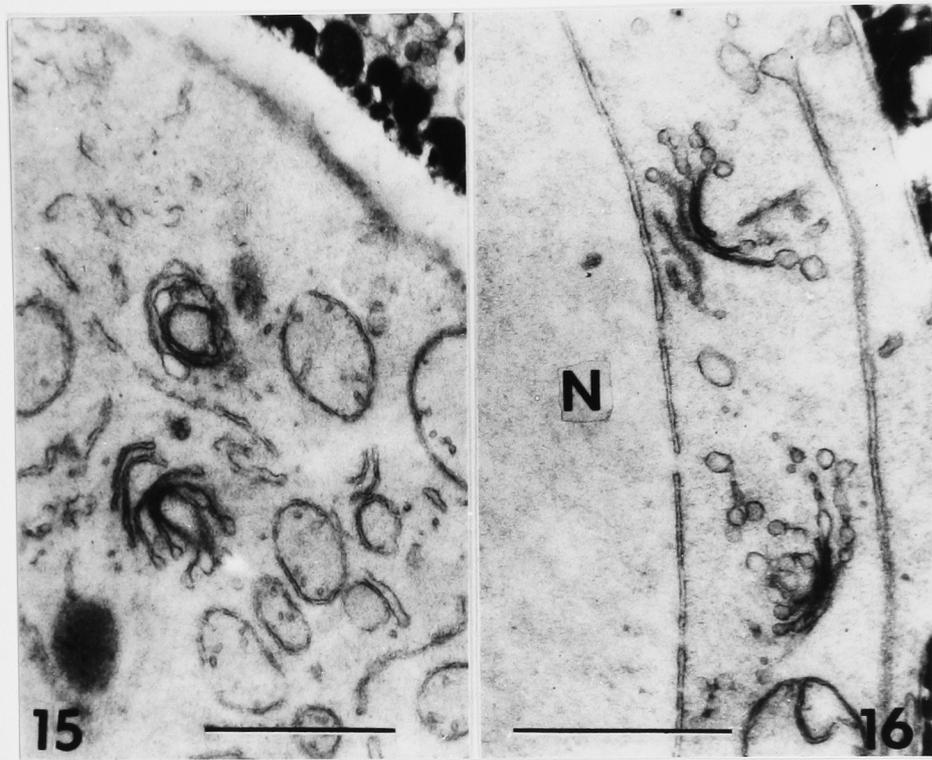
Figure 30. Golgi bodies in a stigma cell of a flower. e, m and d: ring, mature and degenerating forms of the Golgi body, respectively. N: nucleus, W: cell wall. X 18,000.

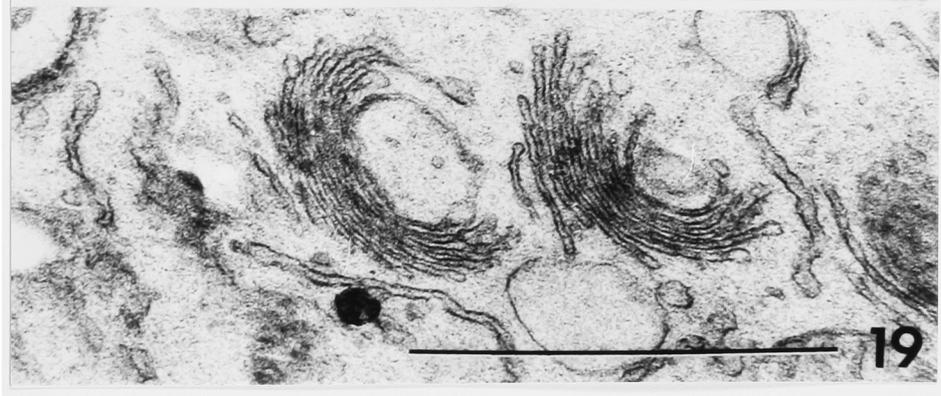
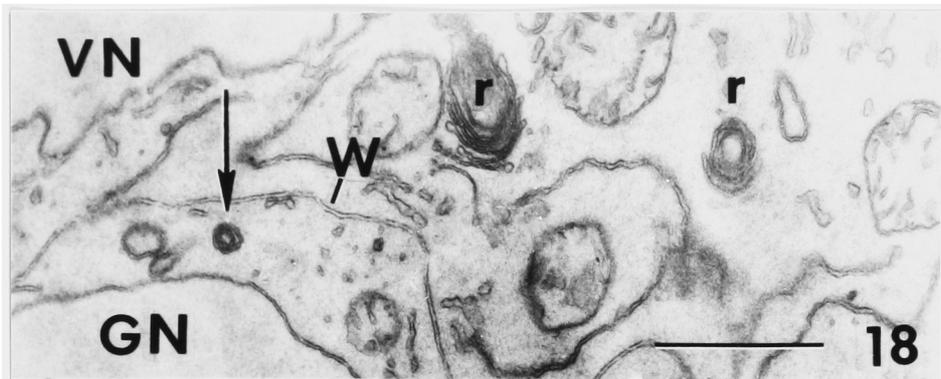
Figure 31. Schematic representation of development of the Golgi body in pollen mother cell (upper row), in microspore after meiosis (middle row) and in pollen grains after postmeiotic mitosis (lower row). Development of the Golgi body is most typical in the pollen grains after postmeiotic mitosis. Developmental pattern of the organelle in the pollen mother cell is essentially similar to that after postmeiotic mitosis. However, the ring-structure does not grow much, and the progress of development of the organelle differs much from one cell to another in one anther loculus. During microspore maturation after meiosis, there is no true mature stage of the organelle, because of early swelling and vesiculation of the cisternae in the ring-structure.

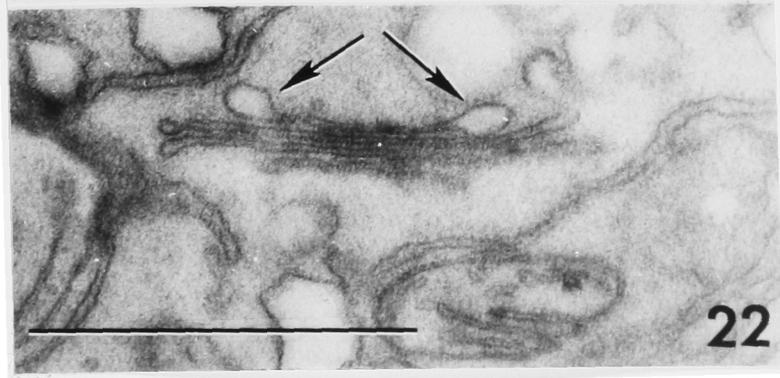
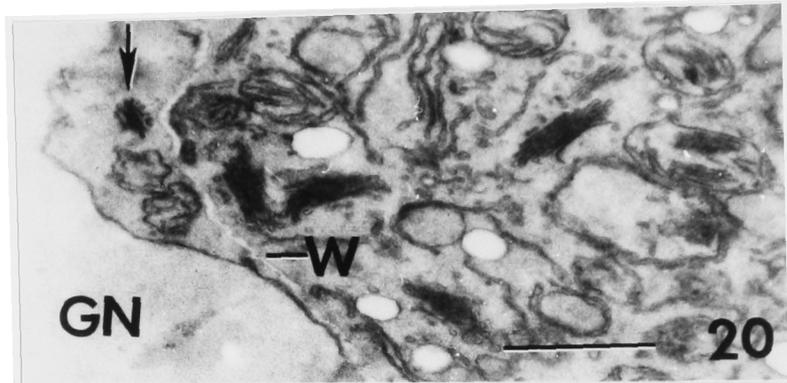


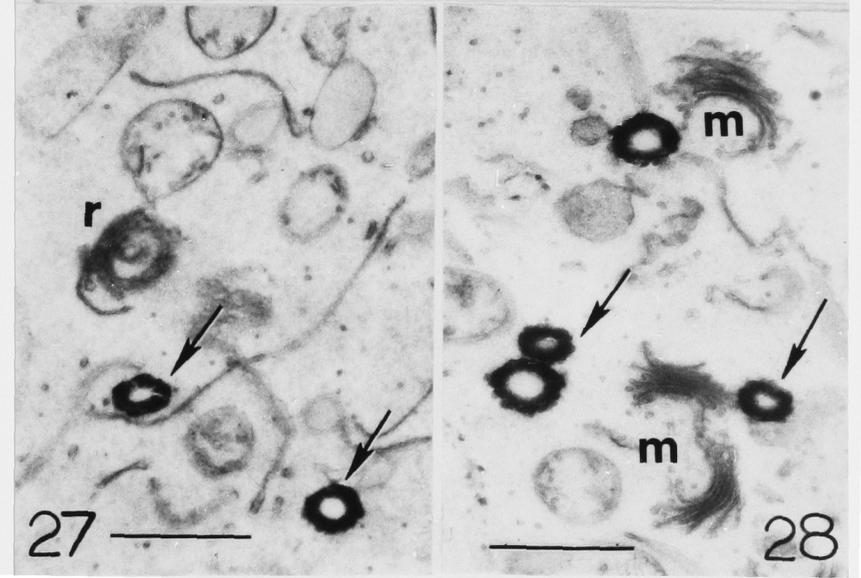
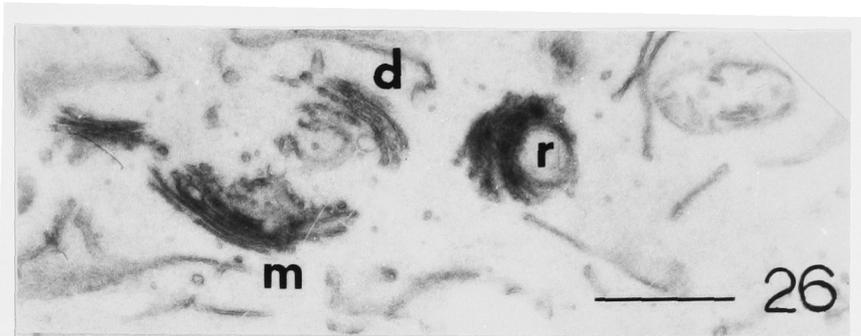


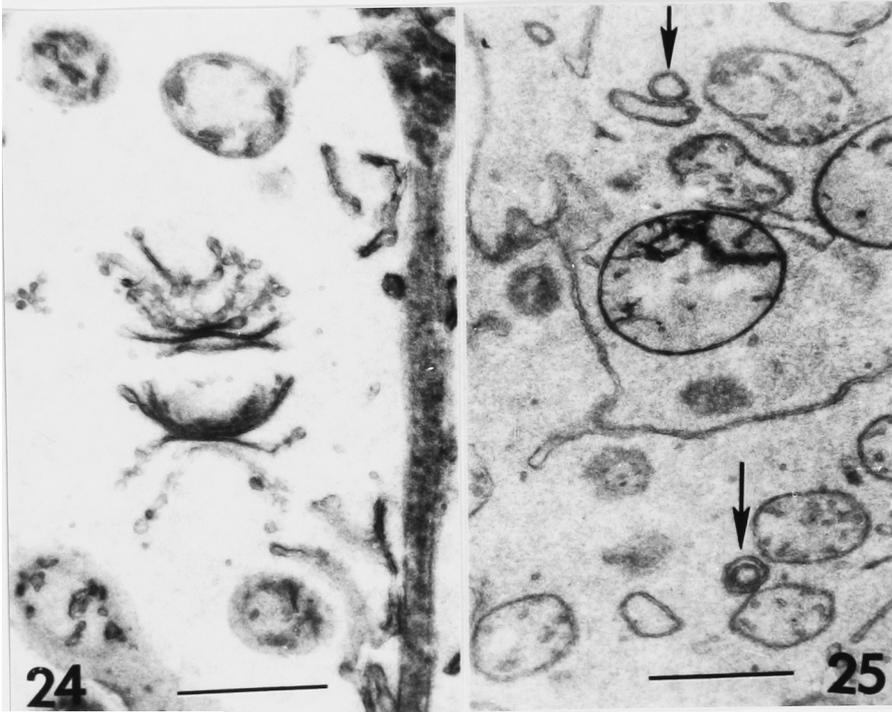
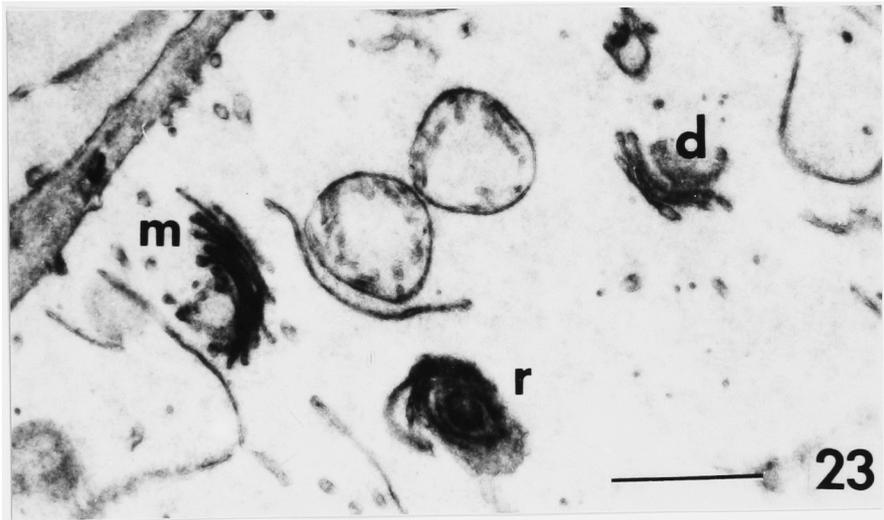












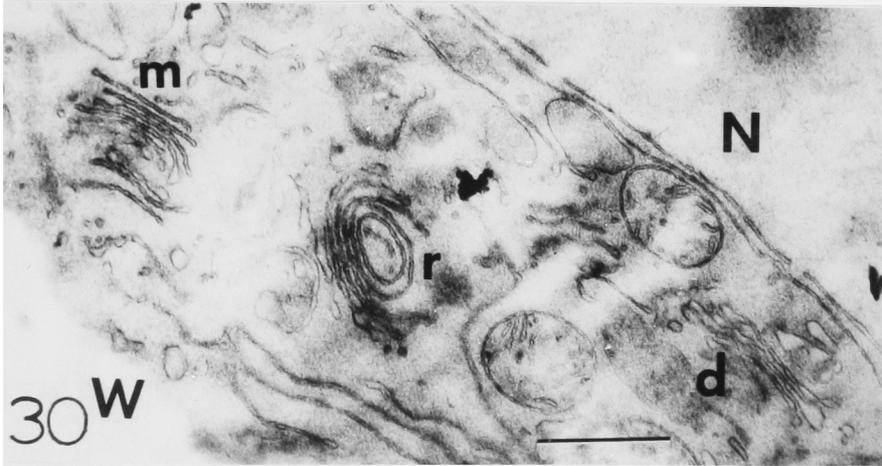
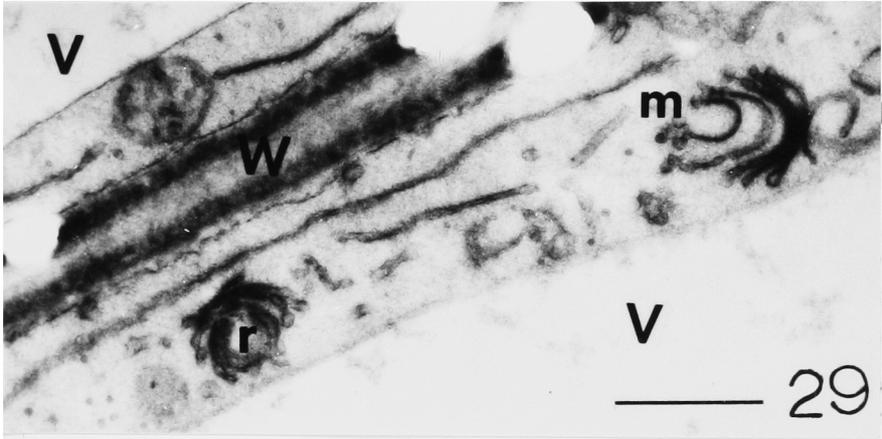


Figure 31

