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The microtubular cytoskeleton during megasporogenesis in the Nun orchid, *Phaius tankervilliae*

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SUMMARY

This study examines the microtubular cytoskeleton during megasporogenesis in the Nun orchid, *Phaius tankervilliae*. The subepidermal cell located at the terminal end of the nucellar filament differentiates first into an archesporial cell and then enlarges to become the megasporocyte. The megasporocyte undergoes the first meiotic division, giving rise to two dyad cells of unequal size. Immunostaining reveals that microtubules become more abundant as the megasporocyte increases in size. Microtubules congregate around the nucleus forming a distinct perinuclear array and many microtubules radiate directly from the nuclear envelope. In the megasporocyte, prominent microtubules are readily detected at the chalazal end of the cell cytoplasm. After meiosis I, the chalazal dyad cell expands in size at the expense of the micropylar dyad cell. At this stage, new microtubule organizing centres can be found at the corners of the cells. The appearance of these structures is stage-specific and they are not found at any other stages of megasporogenesis. The functional dyad cell undergoes the second meiotic division, resulting in the formation of two megaspores of unequal size. The chalazal megaspore enlarges and eventually gives rise to the embryo sac. As the functional megaspore expands, the microtubules again form a distinct perinuclear array with many microtubules radiating from the nuclear envelope. A defined cortical array of microtubules has not been found in *P. tankervilliae* during the course of megasporogenesis.

Key words: nuclear envelope, megasporogenesis, microtubules, *Phaius tankervilliae*, orchid.

INTRODUCTION

In recent years, immunofluorescent techniques and confocal microscopy have revealed the importance of the cytoskeleton during megasporogenesis and megagemetogenesis (Bednara *et al.*, 1988, 1990; Willemse & van Lammeren, 1988; Webb & Gunning 1990, 1994; Huang & Sheridan, 1994). Orchid ovules are small and seem excellent candidates for the study of cytoskeletal elements during reproductive development in plants. The simple integuments of the ovule allow penetration of antibodies after a mild digestion treatment, enabling the detection of cytoskeletal elements (Zee & Ye, 1995; Ye *et al.*, 1996). In our preliminary study of the Nun orchid, *Phaius tankervilliae*, we noted that microtubules are extremely abundant during megasporogenesis and megagametogenesis (Ye *et al.*, 1996). Thus, in order to understand the role of microtubules during megasporogenesis, an extended study was performed using both confocal and transmission electron microscopy. The ultrastructural study confirms that an extensive microtubule array is present in the cell and a majority of microtubules radiate directly from the nuclear envelope.

The Nun orchid follows the Polygonum type of embryo sac development, the most common pattern observed in orchids (Yeung & Law, 1997). Although there has been a large number of light-microscope studies of the Polygonum pattern of embryo sac development in orchids, ultrastructural studies of orchid ovule development are rare (Yeung & Law, 1997). Most information about the ultrastructural aspects of orchid ovule development was obtained decades ago (Yeung & Law, 1997). Therefore, this study also provides a detailed case history of orchid ovule development.

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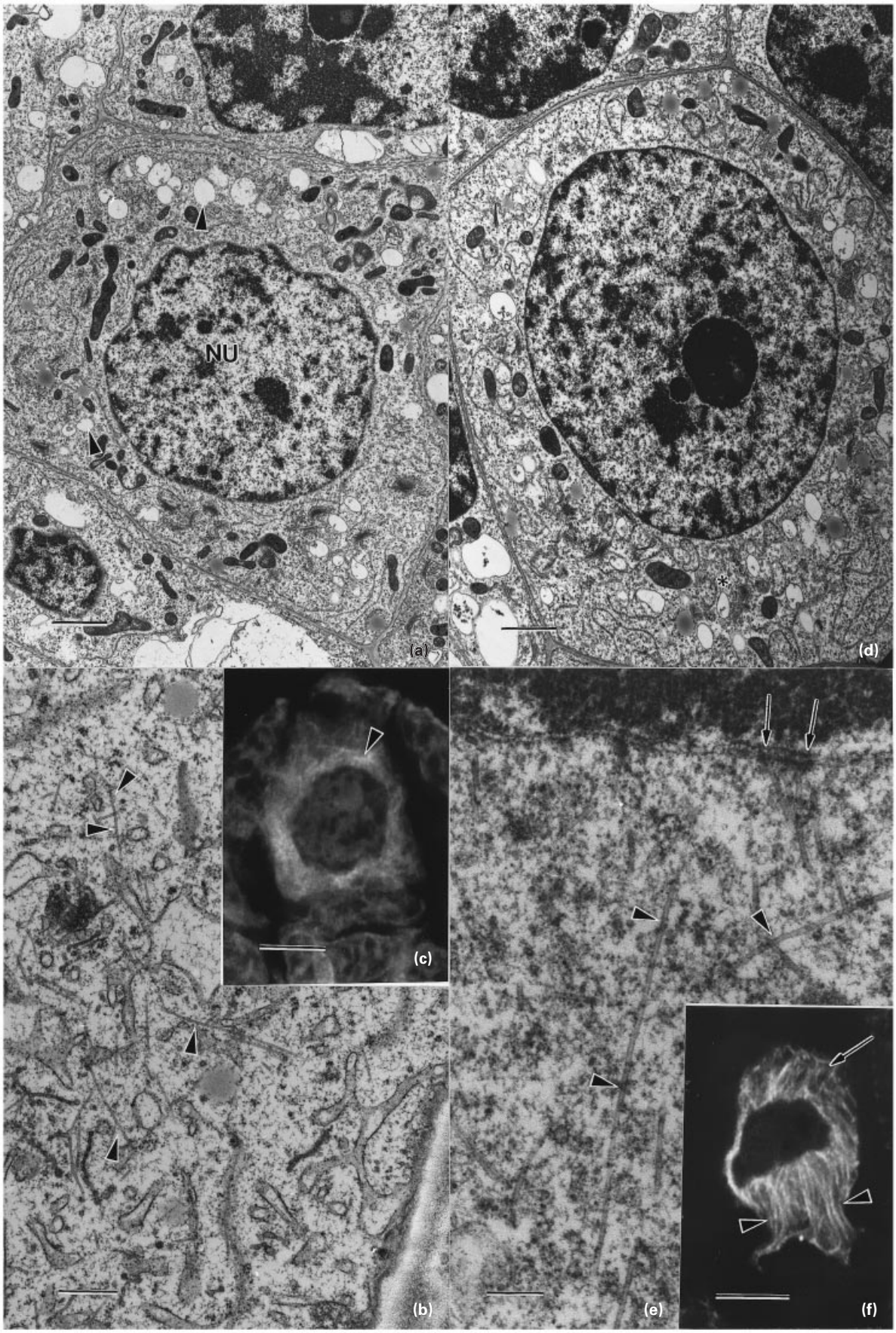

Fig. 1. In the following scanning electron micrographs, the micropylar end of the cell is at the top. (a) In *Phaius tankervilliae*, the hypodermal nucellar cell enlarges and differentiates into an archesporial cell. The nucleus (NU) is the most prominent organelle within the cell. The cytoplasm is rich in organelles. Any vacuoles (arrowhead) are small. Bar, 2 μm. (b) At higher magnification, microtubules (arrowheads) are readily observed

MATERIALS AND METHODS

Plant materials

Flowers and developing fruits of *Phaius tankervilliae* (Aiton) Bl. were collected for structural studies at regular intervals May–July for several growing seasons (1995–98) from hand-pollinated glasshouse-grown plants at the Botany Department, University of Hong Kong. Approximately 20 flowers and fruits were gathered.

Electron microscopy

Fruits were dissected and ovules fixed directly in 2% glutaraldehyde and 2% paraformaldehyde in 0.05 M phosphate buffer, pH 6.8 for 3 h at room temperature. Samples were then washed in the same buffer and post-fixed with 2% osmium tetroxide for another 4 h at room temperature. After post-fixation, samples were washed in the same buffer before dehydration in a graded ethanol series. The ethanol was replaced by propylene oxide before infiltration and embedding with the TAAB 812 epoxy resin.

Ultrathin sections were cut with a diamond knife, using a Reichert Ultracut S ultramicrotome (Leica, Heidelberg, Germany) and stained with a saturated uranyl acetate solution in 50% ethanol and counter-stained with Reynolds lead citrate solution. The sections were observed and photographed using a JEOL 100SX transmission microscope (JOEL, Tokyo, Japan) operated at 80 KV.

In order to select ovules at defined stages of development, the re-embedding method of Kennell & Horner (1985) was used. Serial semithin sections were cut at 3 μm with a glass knife, using a Reichet–Jung Autocut microtome (Leica) and each covered with an empty gelatin capsules with the tip removed. The capsules were glued onto the slides with Permount (Fisher Scientific, Nepean, Ontario, Canada). The epoxy embedding medium was then added to the capsule and allowed to solidify by placing the slides onto a slide warmer. Once secured, the TAAB resin mixture was added to fill the capsules, then polymerized at 60°C for 24 h. The hardened capsules were removed from the slides by pressing sideways at the base with a razor blade. This method allows the removal of a thick section from the slide. The thick section was then sectioned again using an ultramicrotome to obtain 'thin' sections for EM examination.

Immunostaining of microtubules and confocal scanning microscopy

The method of immunostaining of microtubules was similar to that reported for the study of suspensor development in the Nun orchid (Ye *et al.*, 1997). Fruits of *P. tankervilliae* were dissected and ovules were fixed immediately with a 4% paraformaldehyde solution buffered with the microtubule stabilizing buffer, MSB (I), consisting of 100 mM Pipes, 5 mM EGTA, 5 mM $MgSO_4$, pH 6.9, for 1 h at room temperature. After fixation, ovules were washed three times (10 min per wash) in phosphate-buffered saline (PBS). After three washes, ovules were digested with a solution containing 1% cellulase (Sigma, St Louis, MI, USA), 0.2% pectinase (Sigma) and 1% macerozyme (Calbiochem, La Jolla, CA, USA) in MSB (II) buffer, pH 5.6 (2 mM EGTA, 1 mM $MgSO_4$, 0.4 M mannitol and 100 mM Pipes) at 37°C for *c.* 30 min. Each digested sample was attached to a poly-L-lysine (1 mg ml^{-1})-coated coverglass, then a second coverglass was placed on top so that the ovule was slightly flattened. Ovules were subsequently treated with 3% Nonidet P-40 in MSB (II) for 3 h. Samples were rinsed three times (5 min per rinse) in PBS, then treated with $NaBH_4$ (1 mg ml^{-1} in PBS) for 15 min. Before the cells were treated with antibodies, samples were rinsed again in PBS. The preparations were then stained with mouse monoclonal anti-á-tubulin (Sigma T9026, 1:500 dilution) for 2 h at 37°C, then with FITC conjugated anti-mouse IgG (Sigma F0257, 1:16 dilution) for 1 h at 37°C. Samples were washed with PBS between antibody incubation for 30 min (three changes, 10 min for each change). After the second antibody treatment, they were washed in PBS, rinsed in distilled water, and mounted using an antifade mountant (AFT-10, Citifluor, London, UK). The controls were treated in the same way, but without the primary antibody. The preparations were ex-

in the cytoplasm of the archesporial cell. They appear as a reticulate network found throughout the cytoplasm. Bar, 0.5 μm. (c) Immunostaining of microtubules at the archesporial stage. The microtubules are evenly distributed in all parts of the cell and appear as a perinuclear array around the nucleus (arrowhead). There is no distinct cortical array of microtubules. Bar, 10 μm. (d) The archesporial cell enlarges further and differentiates into the megasporocyte. At this early stage of megasporocyte formation, the nucleus is located near the micropylar end of the cell. Increase in the cytoplasm occurs near the chalazal end of the cell (*). With increase in abundance of organelles and the protoplasm, the cell looks dense. Any vacuoles remain small. Bar, 2 μm. (e) At higher magnification, microtubules are readily observed in the cytoplasm and radiating from the nuclear envelope (arrowhead). Where microtubules attach to the nuclear envelope, it appears more electron-dense (arrow). Bar, 0.2 μm. (f) Immunostaining of a developing megasporocyte similar to (d). Prominent microtubules radiate from the nucleus. At the chalazal end of the cell, numerous microtubules (arrowhead) are oriented in parallel with the long cell axis. A more random profile of microtubules is seen at the micropylar end of the cell (arrow). Bar, 10 μm.

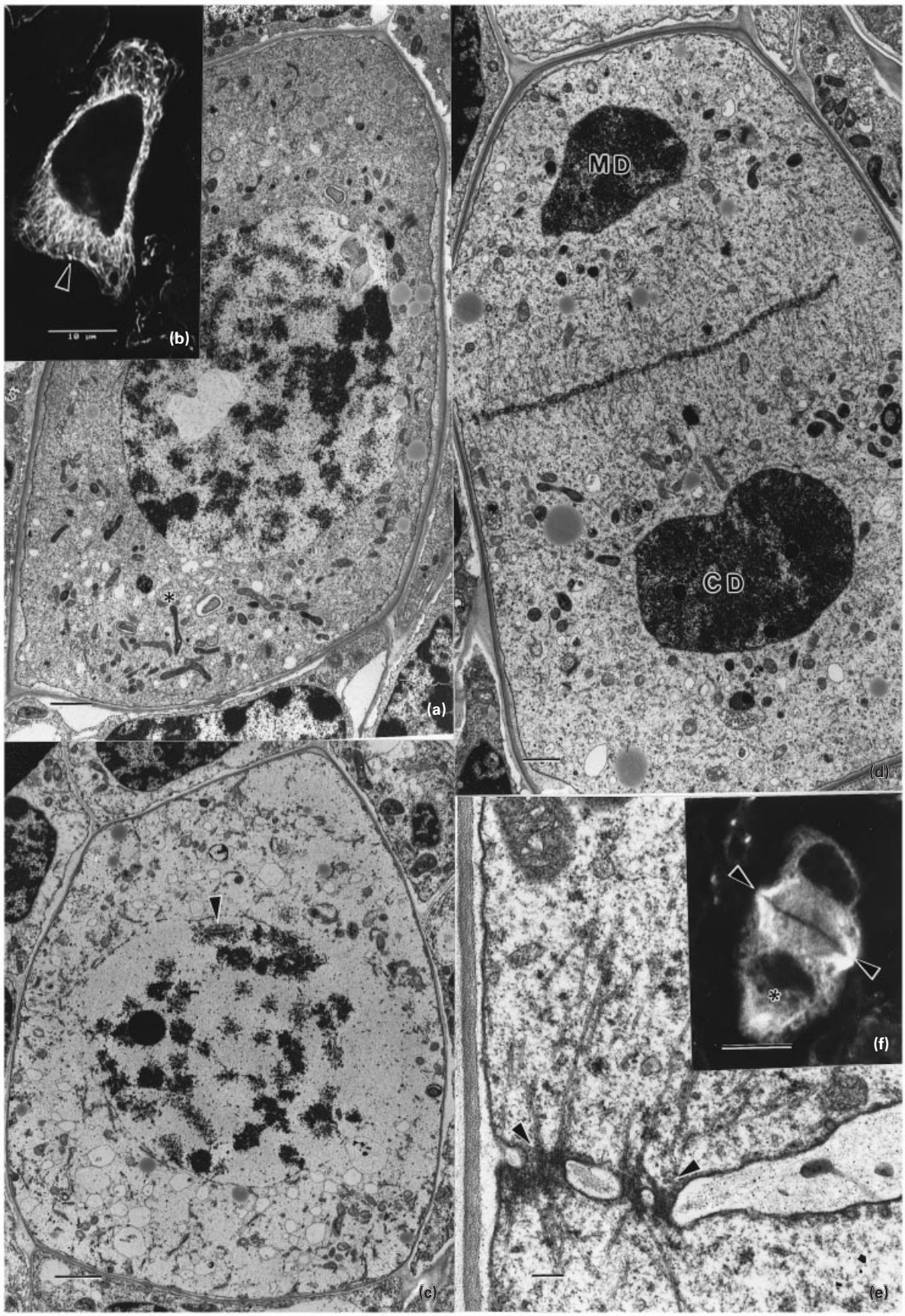

Fig. 2. (a) As the megasporocyte matures, it becomes elongated, with a central nucleus. However, there are more organelles at the chalazal end of the cell (*). Bar, 2 μm. (b) Fluorescence staining of microtubule at a stage similar to (a). A prominent parallel array of microtubules radiates from the nucleus at the chalazal end of the cell (arrowhead). At the micropylar end, the microtubules form a reticulate network in the cytoplasm. Judging from fluorescence intensity, microtubules are not as abundant here. Bar, 10 μm. (c) During meiotic prophase,

amined with a BioRad MRC-600 confocal laser scanning microscope. Images were processed by computer, then photographed and recorded on Kodak T-Max 100 film.

RESULTS

The archesporial cell

The archesporial cell develops from a subepidermal nucellar cell in the nucellar filament. Because it is distinctly larger than surrounding cells, it is readily distinguishable from other cells of the nucellar filament. The cytoplasm of the archesporial cell is rich in organelles (Fig. 1a). Mitochondria, plastids, rough endoplasmic reticulum cisternae, polysomes, and Golgi bodies can be found throughout the cytoplasm. Vacuoles are present within the cytoplasm, however, they are small. The archesporial cell is symplastically connected with neighbouring cells, as plasmodesmata are present in its walls. At higher magnification, many microtubules can be seen throughout the cytoplasm of the archesporial cell (Fig. 1b). Immunofluorescent staining of microtubules confirms the ultrastructural observation of numerous microtubules , evenly distributed throughout the cell and forming a network in the cytoplasm, with a higher concentration around the nucleus (Fig. 1c).

Megasporocyte

The archesporial cell enlarges to form the megasporocyte. Like the archesporial cell, the megasporocyte is rich in organelles (Fig. 1d). At the early stage of megasporocyte development, more organelles are concentrated at the chalazal end of the cell, giving it a polarized appearance (Fig. 1d). Microtubules become more abundant and are easily located in the cytoplasm, many radiating directly from the nuclear envelope (Fig. 1e). The regions of the nuclear envelope where microtubules attach are more electron-dense (Fig. 1e). Immunofluorescent staining confirms and extends the ultrastructural observations. As the archesporial cell differentiates into the megasporocyte, microtubules become more abundant in the cytoplasm. At the early stage of megasporocyte expansion, those at the chalazal end of the cell align in parallel, oriented in the same direction as the long axis of the cell; those at the micropylar end are more randomly distributed (Fig. 1f). Immunostaining of microtubules indicates that the majority is attached to and radiates from the nucleus, especially those at the chalazal end of the cell (Fig. 1f).

The megasporocyte continues to grow because of an increase in cytoplasmic content (Fig. 2a). The cell is highly cytoplasmic, with many organelles, and there are no large vacuoles (Fig. 2a). The mature megasporocyte becomes elongated. The microtubule profile shows distinct differences between the two poles of the cell: a uniform profile of microtubules radiates from the chalazal end of the nuclear envelope towards the cell wall and the pattern at the micropylar end is more random (Fig. 2b).

When the megasporocyte is about to enter metaphase I, the cytoplasmic density of the cell decreases (Fig. 2c), primarily because of a decrease in the ribosome population. Endoplasmic reticulum cisternae appear as short profiles within the cytoplasm (Fig. 2c).

The dyad

The megasporocyte completes meiosis I to form a dyad. As the daughter nuclei appear, microtubules can be found within the phragmoplast (Fig. 2d). Cell division is unequal, resulting in the formation of a slightly larger chalazal and a smaller micropylar dyad cell (Fig. 2d). As the cell plate forms, the number of microtubules increases at the edge of the newly formed cell plate near the parent cell wall (Fig. 2e), resulting in a brighter fluorescence (Fig. 2f). Furthermore, fluorescence intensity within the cytoplasm is greater in the functional dyad cell (Fig. 2f), indicating that differences in the microtubular cytoskeleton already exist at the early stage of dyad formation. As the megasporocyte completes the first meiotic division, cytoplasmic density gradually increases within the dyad as organelles become more abundant (Fig. 2d).

The newly formed cross wall that separates the two dyad cells is thick (Fig. 3a). Aniline blue staining reveals a strong fluorescence, indicating the presence of callose material. Plasmodesmata are absent from this cell wall. The chalazal dyad cell continues to

a synaptenemal complex (arrowhead) can be found within the nucleus and there is a decrease in the overall cytoplasmic density of the cell. Micropylar end of cell uppermost. Bar, 2 μm. (d) At telophase I, a cell plate begins to partition the megasporocyte into a dyad of cells. Cytoplasmic density increases. Cell division is slightly uneven, giving rise to a smaller micropylar dyad cell (MD) and a larger chalazal dyad cell (CD). Bar, 2 μm. (e) Near completion of cell-plate formation, microtubules are readily found at the edge of the newly formed cell plate. Furthermore, at these sites, electron-dense material (arrowhead) appears to associate with the microtubules. Micropylar end of cell uppermost. Bar, 0.2 μm. (f) Fluorescent-staining image of a dividing megasporocyte similar to that in (e). There is bright staining of microtubules near the edge of the cell plate (arrowhead) and the parent cell walls. Furthermore, the chalazal dyad cell (*) has a stronger fluoresence in the cytoplasm, indicating the presence of more microtubules. By contrast, the micropylar dyad cell shows much weaker fluorescence. Bar, 10 μm.

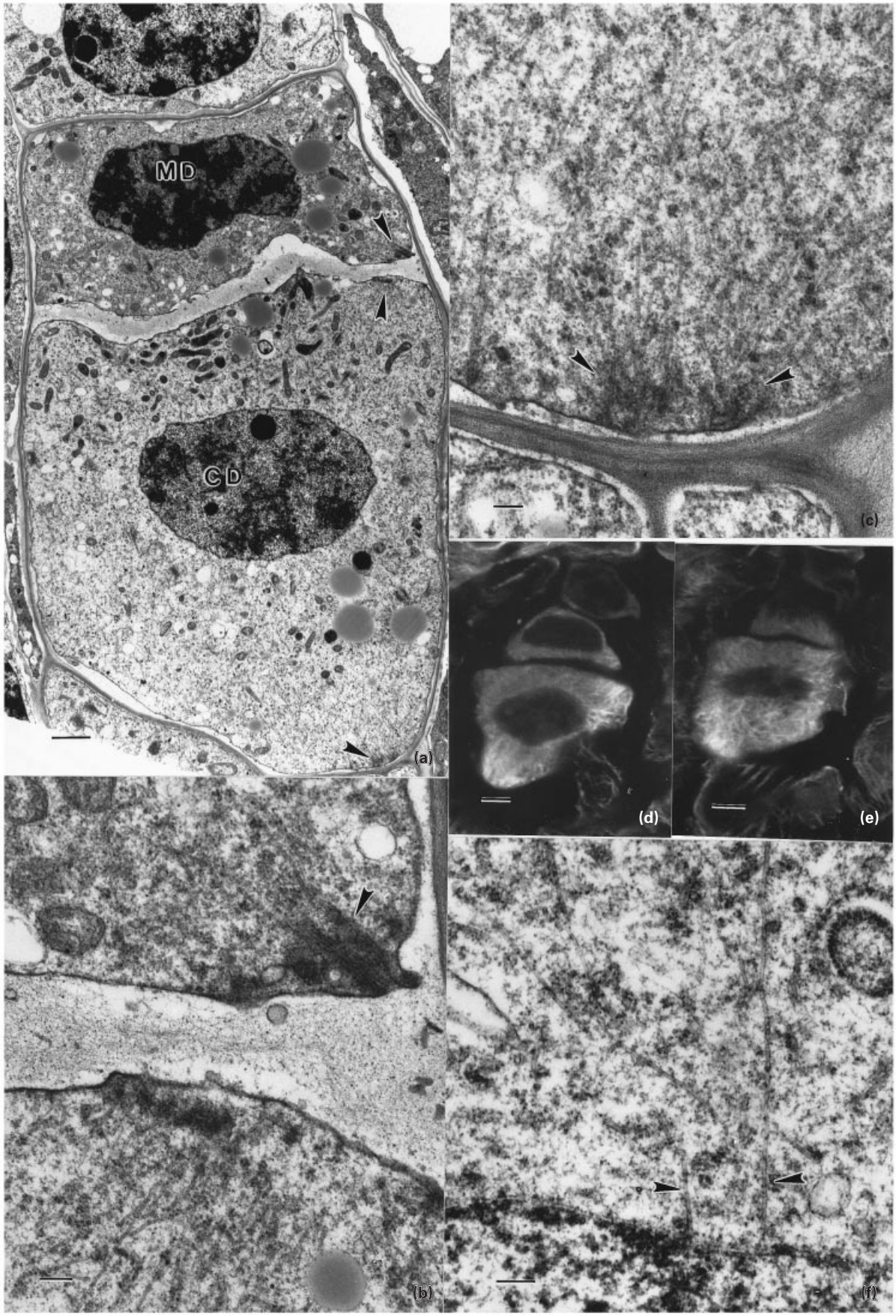

Fig. 3. (a) The dyad stage. A new cell wall forms across the dyad. The chalazal dyad cell (CD) gradually expands and more organelles are located near the newly formed cell wall. The micropylar dyad cell (MD) gradually becomes more electron-dense. The chromatin within the nucleus becomes dense. At this stage only, new microtubule foci (arrowheads) can be found near the corners of the cells. For higher magnification of

grow, while the micropylar dyad cell begins to degenerate (Fig. 3a).

At this stage, besides the nucleus with microtubules radiating from the nuclear envelope (Fig. 3f), new and distinct microtubule foci can be found within the cytoplasm of both dyad cells near the newly formed cell wall separating the dyad (Fig. 3b) and near the end walls of the chalazal dyad cell (Fig. 3c). These microtubule foci are composed of electron-dense amorphous material in which microtubules are embedded (Fig. 3b). Immunostaining of microtubules also confirms ultrastructural observations. In the functional dyad cell, brightly fluorescing regions are found to localize only near the corners of the cell (Fig. 3d,e). As the functional dyad expands, distinct microtubule foci near the cell walls are no longer detectable and are not found at other stages of megaspore development.

At a later stage of dyad development, the micropylar dyad cell becomes more electron-dense and degenerates without undergoing the second meiotic division (Fig. 4a). The cytoplasm of the degenerating micropylar dyad cell becomes very compact and some lipid bodies remain. The chromatin within the nucleus becomes highly condensed (Fig. 4a). In contrast with the degenerated micropylar dyad cell, the functional dyad cell continues to expand. There are numerous organelles, especially at the micropylar end of the cell (Fig. 4a). Microtubules remain abundant within the cell and a majority of the microtubules radiates from the nuclear envelope.

The functional megaspore

The functional dyad cell undergoes meiosis II to produce two megaspores. Cell division is unequal, resulting in a much larger chalazal megaspore and a small micropylar megaspore (Fig. 4b). Most of the cell of the smaller megaspore is occupied by the nucleus, in which there is dense chromatin, a prominent nucleolus and only a few organelles.

The functional megaspore is characterized by a large nucleus. A polar distribution of organelles can still be found at this stage and vacuoles remain small (Fig. 4b). It continues to elongate before embryo sac formation. There are more organelles at the micropylar end of the cell (Fig. 5a). At this stage, microtubules remain very abundant and the majority of them radiates from the nucleus, forming a distinct perinuclear array (Fig. 5b). Just before embryo sac formation, many vacuoles begin to appear within the cytoplasm of the functional megaspore (Fig. 5c), resulting in further growth of the functional megaspore and high compression of the non-functional megaspore (Fig. 5c). Microtubules remain abundant during megagametogenesis (Ye *et al.*, 1996).

DISCUSSION

Orchid ovules develop from nucellar filaments, which consist of a column of 3–7 nucellar cells covered by a single layer of epidermis. At the completion of megasporogenesis, the functional megaspore is protected by a layer of epidermis and thin integuments (Yeung & Law, 1997). The relative ease of microtubule staining within the ovule of *P. tankervilliae* is probably due to its simple organization. The optimized protocol used in this study allows for a better penetration of antibodies into the cells, hence better staining of microtubules.

Phaius tankervilliae undergoes the monosporic pattern of embryo sac development. The pattern of ultrastructural changes is essentially similar to those of other angiosperm species (Huang & Russell, 1992). The most significant finding of this study is that microtubules are extremely abundant in the cytoplasm of the cell from differentiation of the megasporocyte. Furthermore, the majority of the microtubules congregates around the nucleus and radiates from the nuclear envelope, forming a prominent perinuclear array of microtubules. There is no clearly defined array of microtubules in the cortex.

In the few reports on microtubule organization during megasporogenesis (*Arabidopsis thaliana* (Webb & Gunning, 1990, 1994), *Gasteria verrucosa* and *Chamaenerion angustifolium* (Bednara *et al.*, 1988), *Zea mays* (Huang & Sheridan, 1994), and *Cymbidium sinense* (Zee & Ye, 1995)), a distinct perinuclear array with microtubules radiating from the nucleus has been observed at some stage of development, although different microtubular arrays have been found.

The potential importance of the nucleus and the perinuclear microtubule-organizing centre leads to the proposal of the 'cell body' concept in animal cells by Mazia (1993) (see also Baluska *et al.*, 1998). The 'cell body' is composed of the nucleus/centrosome complex and also includes the different arrangement of microtubules generated by the

these structures, see (b,c). Bar, 2 μm. (b) Microtubule foci, composed of electron-dense amorphous material which bind to groups of microtubules (arrowhead), are located near the corners of the newly formed cell wall. Bar, 0.2 μm. Micropylar end of cell uppermost. (c) Similar microtubule foci (arrowhead) at the chalazal end of the functional dyad cell. Bar, 0.2 μm. (d,e) Optical sections through a dyad, confirming the presence of microtubule foci at the corners of the newly formed dyad cells. There are more microtubules in the functional dyad cell. Micropylar end of cell uppermost. Bars, 5 μm. (f) In addition to those radiating from the new microtubule foci near the corners of the cells, microtubules (arrowheads) are also found to radiate from the nuclear envelope. Micropylar end of cell uppermost. Bar, 0.2 μm.

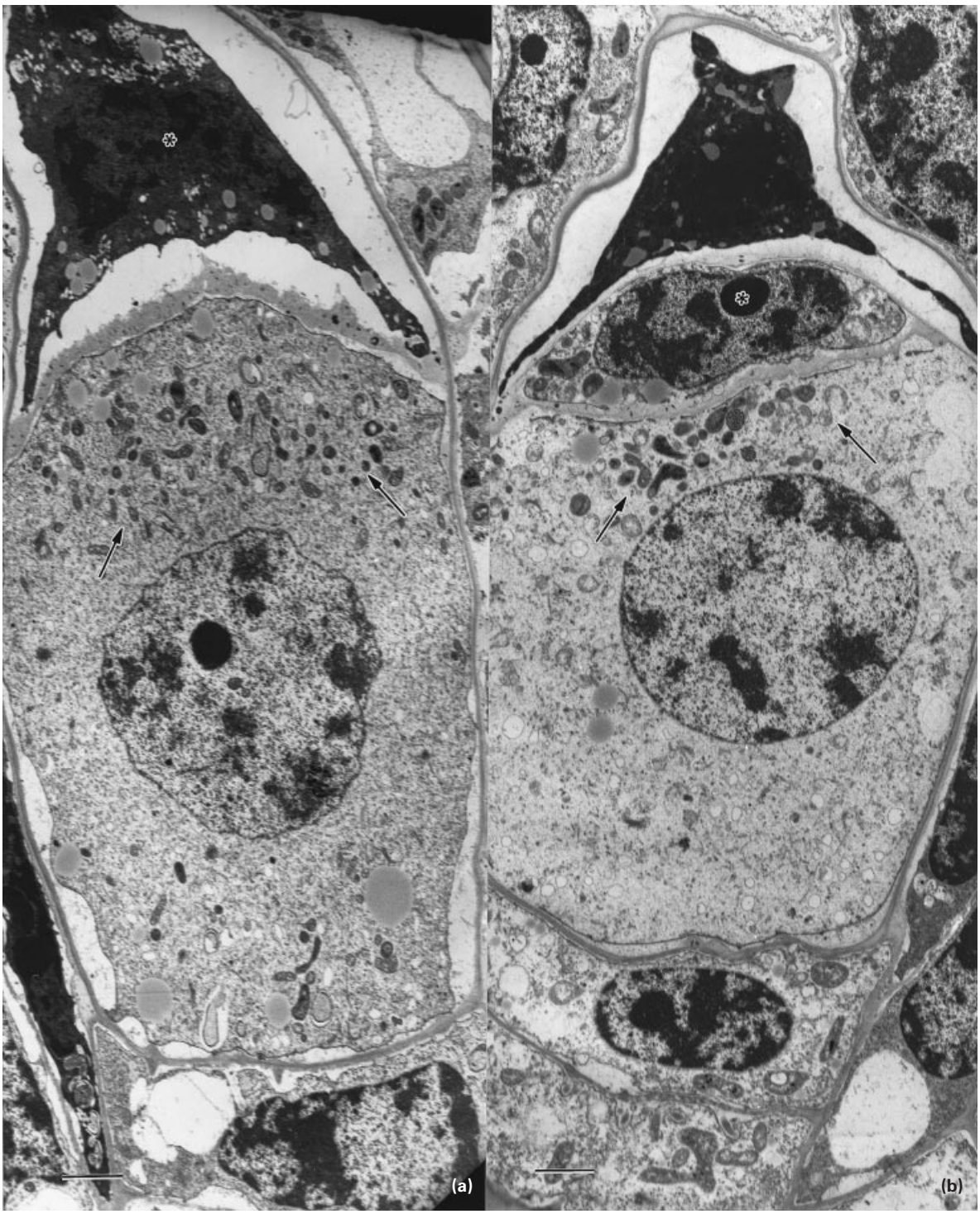

Fig. 4. (a) The functional dyad cell expands at the expense of the micropylar dyad cell (*). A polar distribution of organelles (arrows) can be found in the cytoplasm of the functional dyad cell. Bar, 2 μm. (b) The second meiotic division occurs rapidly and results in two megaspores of different size. In the smaller megaspore (*), the cell is occupied by a nucleus and a few organelles in the cytoplasm. In the larger, functional megaspore, a higher concentration of organelles (arrows) remains near the micropylar end of the cell. Bar, 2 μm.

centrosomes (Mazia, 1993). Baluska *et al.* (1998) further extended the 'cell body' concept to include plant cells. Changes in the 'cell body' (i.e. the nucleus and associated microtubules) could allow for and regulate a variety of cellular processes during growth and development (Baluska *et al.*, 1998). In *P. tankervilliae*, the absence of a prominent cortical array of microtubule suggests that the 'cell body' could play a key role in regulating the process of megasporogenesis.

In *Phaius tankervilliae*, a randomly arranged nuclear array of microtubule pattern is observed at the archesporial stage. A similar pattern is found in *Gasteria* at the same stage (Willemse & Van Lam-

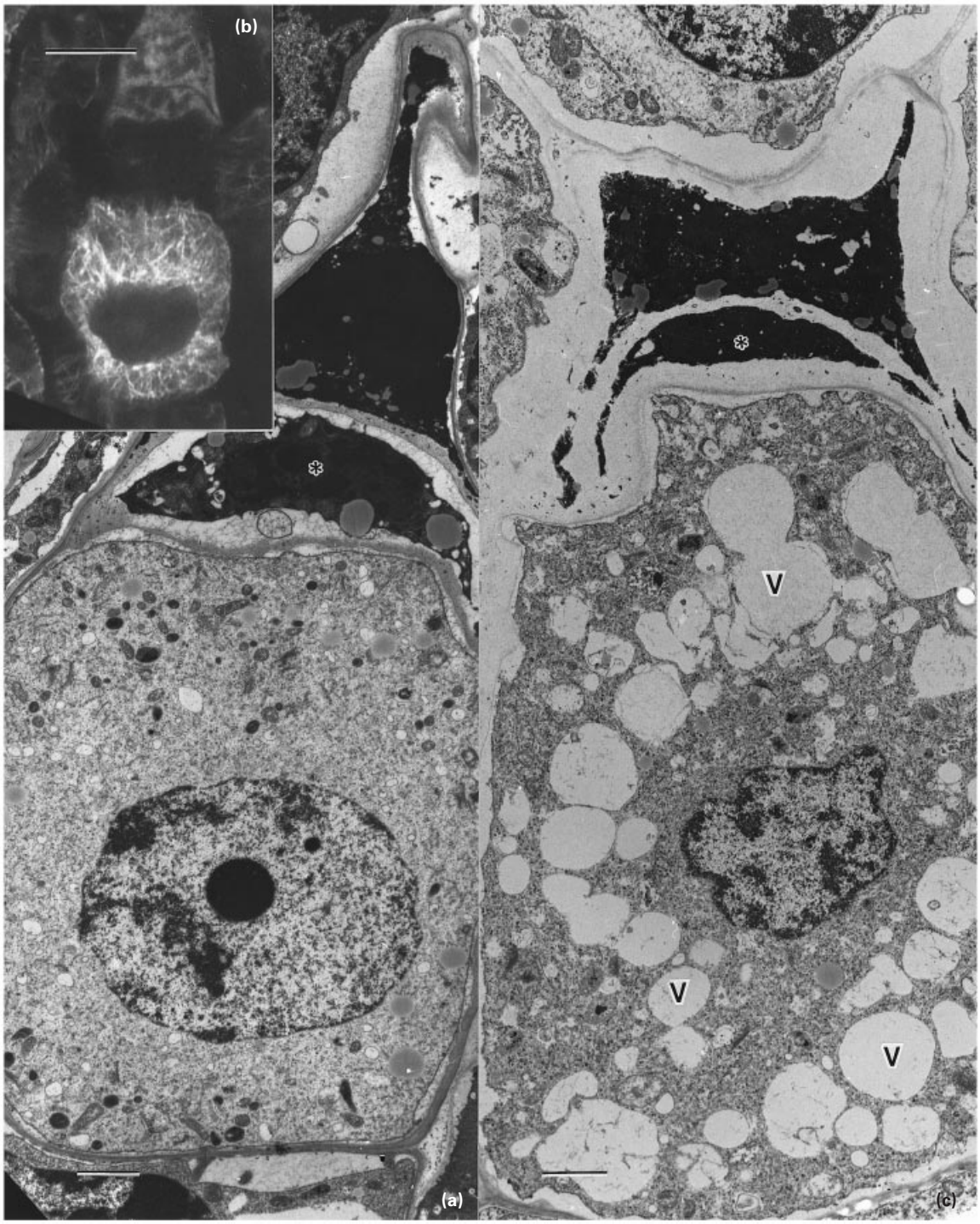

Fig. 5. (a) The functional megaspore continues to expand while the nonfunctional megaspore (*) degenerates and becomes very electron-dense. Growth of the cell is caused by the increase in cytoplasm and not vacuolation. There are a few, small, vacuoles. Micropylar end of cell uppermost. Bar, 10 μm. (b) Immunostaining of microtubules at a stage similar to (a). The pattern of staining remains the same, with microtubules forming a perinuclear array radiating from the nuclear envelope. Bar, 2 μm. (c) The mature megaspore expands further because of vacuolation. Many vacuoles (V) begin to appear throughout the cytoplasm in preparation for embryo-sac formation. The nonfunctional megaspore (*) becomes highly compressed. Micropylar end of cell uppermost. Bar, 2 μm.

meren, 1988). As the megasporocyte begins to form, the abundance of microtubules greatly increases,with more at the chalazal end of the cell. Furthermore, these microtubules tend to orient in parallel with the long axis of the cell; however, those at the micropylar end tend to be more randomly arranged within the megasporocyte. The microtubule profile suggests several potential functions during megasporogenesis. First, the longitudinally aligned microtubules correlate well with the elongation of the megasporocyte

and the functional dyad cell suggesting that they play a role in cell elongation. Second, during the course of megaspore formation, organelles are not evenly distributed within cells. For example, at the dyad stage, more organelles are found at the micropylar end of the functional dyad cell (Fig. 4a) and this pattern is maintained after the second meiotic division (Fig. 4b). Thus there must be a cytoplasmic framework which maintains organelle position. It has been suggested that the perinuclear array of microtubules could maintain the cytoplasmic organization of the cell, especially position of organelles and nucleus (Webb & Gunning, 1994). Our study clearly indicates that they can play such a role during the course of megasporogenesis Third, during the course of megaspore formation, two unequal cell divisions occur. We have not encountered a preprophase band during our investigation. One of the suggested functions of the microtubules is aiding the translocation and distribution of macromolecules within cells (Webb & Gunning, 1994). The difference in the pattern of microtubule arrangement between the micropylar and chalazal end of the cell might result in difference in macromolecule distribution, which could provide the necessary signal for the unequal cell division.

The observation that the nuclear envelope acts as the microtubule nucleation site has been reported in different studies (Lambert *et al*., 1991). In the study of plant reproductive processes, the radial arrangement of microtubules around the nucleus in *Lilium* microspores is clearly documented by Dickinson & Sheldon (1984). Microtubules radiating from the nucleus have been observed in the megaspores of a number of plants (Bednara *et al*., 1988; Brown & Lemmon, 1988; Willemse & Van Lammeren, 1988; Webb & Gunning, 1990; Huang & Sheridan, 1994; Zee & Ye, 1995; Tung, 1997). Nuclear-envelope-associated microtubules are very conspicuous in the megasporocyte, dyad, and the chalazal megaspore of *P. tankervilliae*. In this study, ultrastructural observations have confirmed that the nuclear envelope can serve as the nucleation site for microtubules. At the sites where microtubules attach, the nuclear envelope is more electron-dense, suggesting that nucleating material has been incorporated in it. The ultrastructural feature of microtubule attachment in *P. tankervilliae* is similar to that reported in *Lilium* (Dickinson & Sheldon, 1984). Mizuno (1993) shows that nuclei of tobacco cells contain material like that of a microtubule organizing centre (MTOC) which when incorporated into the nuclear envelope at defined stages might enable it to serve as a MTOC. Differences in distribution of MTOC material in different parts of the cell will be important in regulating cell morphogenesis.

Traditionally, microtubules are found to radiate from centrosomes of animal cells and from an amorphous structure near the mitotic apparatus in plant cells. It is clear from many recent studies that other sites can also become nucleating sites for the microtubule pattern (Baluska *et al*., 1997; Hyman & Karsenti, 1998; Vaughn & Harper, 1998). In *P. tankervilliae*, in addition to the nuclear envelope, which could serve as the MTOC, new microtubule foci have been found only at the early dyad stage near the walls at the corners of the cells and are composed of an electron-dense amorphous material. A similar substance has been reported in other MTOC systems, in plants such as *Azolla* (Gunning, 1980). The origin of these microtubule foci is still unknown. However, Hasezawa & Nagata (1993) have provided evidence to show that a protein which is responsible for the initiation of microtubule formation is able to move from the surface of the nuclear envelope to the cell cortex. The presence of additional MTOCs at this stage coincides with the expansion of the functional dyad cells. The additional microtubules forming at the corners of the cell might aid the generation of forces necessary for cell expansion.

In conclusion, the abundance of microtubules indicates the importance of these organelles during megasporogenesis. The nuclear envelope serves as one of the MTOCs during megasporogenesis and additional MTOCs appear transiently during dyad formation. The dynamic interactions between nuclear envelope, MTOC material and microtubules could influence the developmental fate of a cell. Besides microtubules, the other major components of the cytoskeleton, actin filaments, probably also play an important role. Further studies on orchids will provide additional insight into the female reproductive process.

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