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Microtubule organization in the differentiating transfer cells of the placenta in *Lilium* spp.

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Summary. Placental cells in the ovarian transmitting tissue of *Lilium* spp. are organized as transfer cells with inbuddings facing the ovarian locule. A detailed analysis of microtubule (MT) organization during development of these polarized cells is reported here. Formation of wall projections occurs at the apical part of the cell starting on the day of anthesis, and a fully mature secretion zone is found four days after anthesis. MTs are organized into distinct cortical and central arrays. The cortical array undergoes a unique transition at anthesis. MTs in the basal half of the cell remain in longitudinal bundles while in the apical half of the cell their longitudinal orientation is replaced by a transverse alignment. One day after anthesis, these transverse bundles become a meshwork of short, randomly organized MTs, while MTs in the basal half of the cell retain their longitudinal alignment. The realignment of MTs in the apical half of the cell coincides with the deposition of the secondary cell wall. The central array is composed of short, randomly arranged strands of MTs in the cytoplasm between the nucleus and the apical and basal periclinal walls of the cell. This array first appears as solitary strands in the apical part of the cell one day before anthesis. The central array extends during development and is eventually seen in the basal half of the cell. We propose that MTs in the cortical region near the apical wall act as templates for the deposition of cellulose microfibrils in the secondary cell wall. MTs in the central array in these transfer cells may be involved in the trafficking of vesicles and/or positioning of organelles near the secretion zone.

Keywords: Microtubules; Placental cells; Transfer cells; Transmitting tissue; *Lilium longiflorum*.

Abbreviations: MT microtubule; daa day after anthesis; dba day before anthesis.

Introduction

In *Lilium* spp., cells forming an epithelium on the placenta constitute the ovarian transmitting tissue (Singh and Walles 1992). The secretory products of these cells are vital for the continued growth of the pollen tubes in the ovary. The tubes grow towards the micropyle in this exudate, which probably provides nutrition and chemotropic guidance to the tubes (Welk et al. 1965; Tilton and Horner 1980; Kronestedt et al. 1986; Van Roggen et al. 1988; Janson et al. 1993, 1994). The placental cells are papilla-shaped with a domed apex. The wall facing the locule has prominent wall ingrowths (Singh and Walles 1992, 1995), characteristic of a transfer cell (Gunning and Pate 1969). These polarized wall invaginations provide an effective system for the transport of solutes to the ovarian locus by increasing the plasma membrane surface area. In *Lilium* spp., the complex labyrinth of the transfer cell wall along with its associated structures is termed the secretion zone (Rosen and Thomas 1970).

There is a basipetal progression of chemotropic activity in the pistil of *Lilium* spp. It begins at the stigmatic papillae 3–5 days before anthesis, followed by the style 2–3 days before anthesis, and the ovary 2 days after anthesis (Welk et al. 1965). Our study of the sequential changes during morphogenesis in the ovary (Singh and Walles 1992) showed that the structural maturation of the transfer cells correlates with the basipetal pattern of functional maturity observed by Welk et al. (1965). The main structural feature in the differentiation of the placental cells is a rapid and conspicuous change in the cell wall in the secretion zone. The cell wall develops from a thin layer into a complex maze of invaginations, several micrometers deep. In a detailed examination of ovarian-transmitting-tissue differentiation in *L. regale* we found that wall ingrowth

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development begins on anthesis day and the secretion zone attains its maximum depth four days after anthesis (Singh and Walles 1995).

The sequential laying down of the cell wall requires the presence of microtubules (MTs) to control the alignment of newly synthesized cellulose microfibrils (for reviews, see Williamson 1991, Cyr 1994, Wymer et al. 1996). MTs guide the cellulose-synthesizing complex across the face of the plasma membrane so that new microfibrils reflect the precise arrangement of the underlying MTs (Giddings and Staehelin 1988, 1991). However, a recent model proposes a bidirectional flow of information where the cellulose microfibrils and, hence, cell elongation provide spatial cues for cortical MT organization (Fisher and Cyr 1998).

In cells with nonuniform secondary walls, cellulose deposition is controlled by the localized assembly of MTs. This is seen in developing xylem tracheary elements (Falconer and Seagull 1986, Hogetsu 1991, Funada et al. 1997) and differentiating guard cells (Cleary and Hardham 1989, Marc et al. 1989, McDonald et al. 1993). Transfer cells also have a nonuniform deposition of secondary-cell-wall material in the form of cell wall ingrowths. Most studies on the cytoskeleton in cell wall ingrowths have examined the filiform apparatus in synergids (Huang and Russell 1994, Ye et al. 1996). Recently however, Bulbert et al. (1998) examined MT organization in transfer cells in the cotyledons of *Vicia faba* embryos. These authors report that in immature epidermal cells of the cotyledons, the MTs are localized throughout the cortex, but in more mature cells with wall ingrowths, MT organization is polarized and concentrated in cortical regions adjacent to the wall protuberances. They concluded that the unique distribution of MTs is required for a polarized distribution of cell organelles in these cells and that the MTs play no direct role in the buildup of the cell wall ingrowths (Bulbert et al. 1998). In the present study, we investigate with confocal microscopy the detailed organization of MTs in a different transfer cell system, the developing placental cells in ovarian transmitting tissue of *L. longiflorum*.

Material and methods

Material

Pistils from 1 day before to 8 days after anthesis were harvested from *Lilium longiflorum* flowers obtained from commercial sources. The ovary was cut open longitudinally and a thin layer of placenta was dissected out.

Confocal microscopy

The placental layer was fixed with 4% paraformaldehyde in PEM buffer (100 mM piperazine-N,N′-bis(2-ethanesulfonic acid), 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, and 1 mM $MgSO_4$) at pH 7.0 for 1 h at 20 °C with slow agitation. Cells were then rinsed in PEM at pH 5.0 and transferred to an enzyme solution of 0.5% cellulase, 0.5% macerase, with 4 mM phenylmethylsulfonyl fluoride, 20 µg of Leupeptin, 10 µg of Pepstatin, and 50 µg of Aprotinin per ml in PEM buffer (pH 5.0). Cell walls were digested with this enzyme mixture at 35 °C for 1 h, with strong agitation. The tissue was then rinsed in a blocking buffer (phosphate-buffered saline, pH 7.5, plus 1% bovine serum albumin and 1% Triton X-100) and incubated overnight at room temperature with mouse monoclonal antibodies to α-tubulin (Amersham), diluted 1 : 50. Afterwards, the tissue was rinsed in blocking buffer and incubated for 2 h at 35 °C with sheep anti-mouse IgG conjugated to Cy-3, diluted 1 : 200 and rinsed again in blocking buffer. The tissues were moved to a slide with a drop of Moviol and gently squashed under a cover slip to release the placental cells. Controls, omitting either the primary antibody or the primary and secondary antibodies, were processed in parallel with treated tissues. Samples were examined on a Zeiss LSM510 laser scanning confocal microscope with optical sections of 350 to 400 nm.

Transmission electron microscopy

Samples of *Lilium regale* were prepared for transmission electron microscopy as previously described (Singh and Walles 1995).

Results

Placental cells from one day before anthesis (dba), anthesis day, and 1, 2, 4, or 8 days after anthesis (daa) were used to evaluate MT organization during placental cell development. Placental cells from 1 dba are small. The characteristic papilla shape has not developed and the nucleus dominates the cell volume (Fig. 1 b). The cytoplasm is seen as a narrow band around the nucleus in the basal half of the cell. Secretion has commenced at this stage, and secretory products are seen as small blobs on the outer surface of the wall in the apical part of the cell (Fig. 4). The cuticle is sloughed off from the cell wall but there are no protuberances on the cytoplasmic surface of the wall (Fig. 4). Microtubules in the cell cortex are arranged in oblique or longitudinal arrays, along the long axis of the cell (Fig. 1 a). They are found beneath the apical wall of the cell, but their orientation is very difficult to decipher. A projection of the cell viewed from the base, indicates that MTs form longitudinally aligned loops around the base. Median optical sections indicate that MTs are occasionally present in the noncortical cytoplasm in the apical half of the cell (Fig. 1 b). Since this part of the cell is dominated by a central vacuole, the MTs are not abundant.

On anthesis day the placental cells still possess a thin apical wall, but small amounts of wall material are deposited on the inside of the cell wall (Fig. 5). The cells are slightly more elongate and are developing the typical papilla shape. The apical portion of the cell elongates, while the nucleus remains in the base of the cell and still occupies a large portion of the volume (Fig. 2b). At this stage there is a dramatic change in the orientation of the cortical MTs. In the basal half of the cell they retain their longitudinal orientation, but in the apical half the MTs are transversely arranged (Fig. 2a). There is a transition region between the basal and apical half of the cell as MTs shift from longitudinal to transverse. The labelling at the apical wall is brighter, but any MTs present at this position were too few to discern a distinct pattern. The longitudinal loops at the base of the cell remained intact. In median optical sections, numerous MTs are found in a random orientation in the noncortical cytoplasm of the apical half of the cell (Fig. 2b).

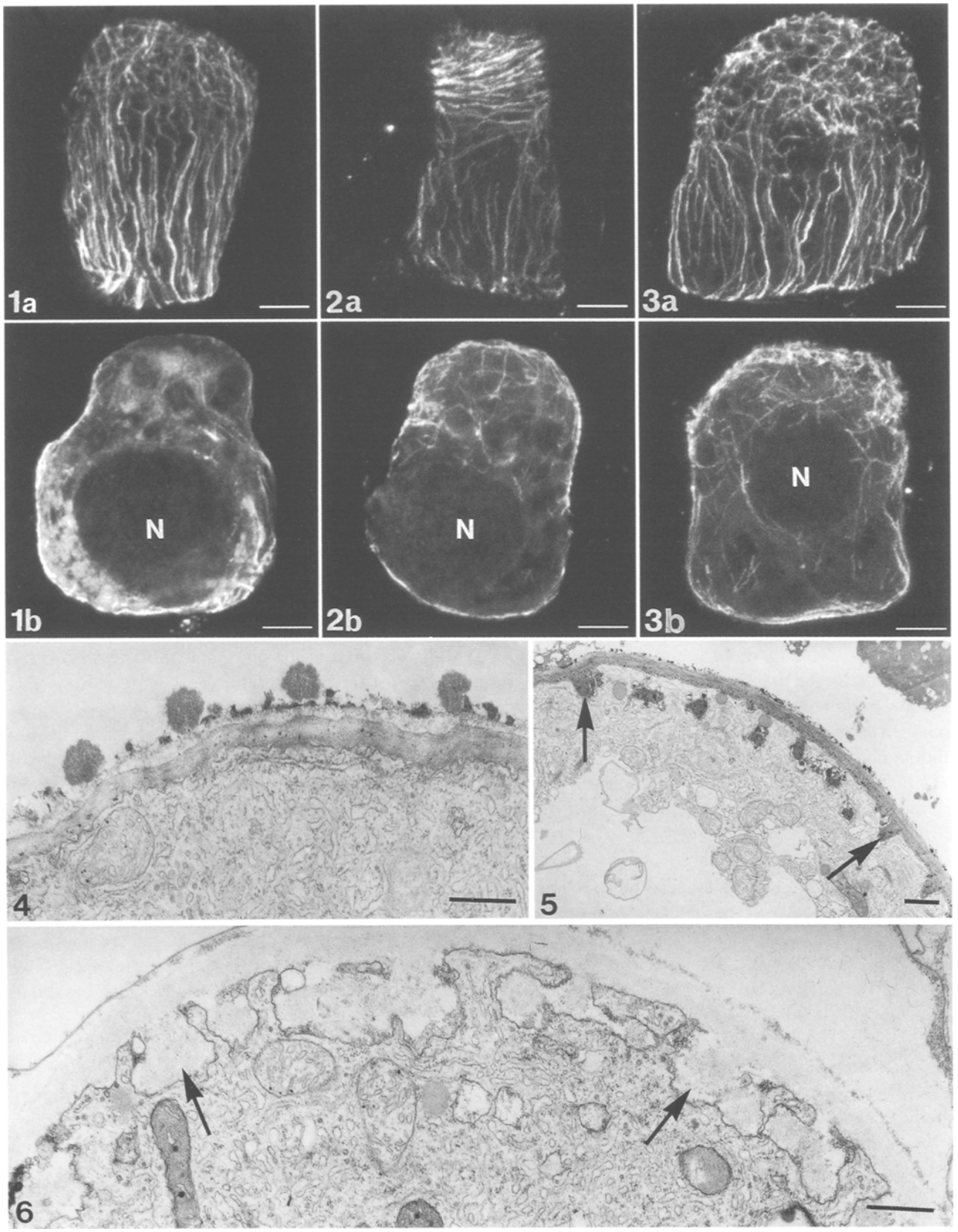
By 1 daa, the cells have attained the typical papilla shape. The beginnings of complex cell wall protuberances are seen (Fig. 6). Both the basal and the apical halves of the cell have expanded, although the expansion in the apical half is more apparent. The nucleus has moved to a slightly more central position. Cortical microtubules in the basal part of the cell remain in longitudinal to oblique bundles. In the apical half MTs are no longer in transverse bundles but are organized in a random network (Fig. 3a). In median optical sections, MTs juxtaposed to the apical dome-shaped cell wall are more distinct and can be distinguished as bright short strands, randomly arranged (Fig. 3b). Random arrays of MTs are seen criss-crossing the cytoplasm between the nucleus and the apical wall. A few MT strands are seen even in the basal portion of the cell (Fig. 3b).

By 2 daa the cell wall at the apical dome is more than twice as thick as the original wall. The wall projections protrude into the cytoplasm and are outlined by the plasma membrane (Fig. 10). The nucleus occupies the same position as in the previous stage. Cortical MTs in the basal half of the cell remain in longitudinal or oblique bundles, while in the apical half of the cell they are even more disordered, forming a network of short criss-crossing MTs (Fig. 7a). At the periphery of the domed cell wall, strands of MTs protrude into the cell wall convolutions outlining the differentiating secretion zone (Fig. 7a, b). In median optical sections, the layer of MTs beneath the growing periclinal wall has increased and the MTs are more concentrated in the cytoplasm between the apical cell wall and the nucleus, forming a random array. MTs in the basal portion of the cell are also more abundant (Fig. 7b).

By 4 daa the placental cell is fully mature, with a well developed secretion zone. The wall labyrinth attains its maximum depth by this stage. A large volume of exudate has accumulated under the cell wall thickenings, pushing the plasma membrane inwards (Fig. 11). Microtubule organization throughout the cell cortex is the same as two days after anthesis, but more MTs radiate out into the mature secretion zone (Fig. 8a, b). MT bundles are often located in deep invaginations of cytoplasm, between the cell wall ingrowths (Fig. 14). The dense band of MTs is still juxtaposed to the secretion zone. Median optical sections reveal a heavy concentration of MT bundles in the apical cytoplasm beneath the secretion zone and MTs are abundant around the nucleus in the basal half of the cell (Fig. 8b).

By 8 daa the petals begin to wilt. The stigma is still wet with secretion. The ultrastructure of the secretion zone is unchanged from 4 daa and there is no increase in the thickness of this zone, but the accumulated exudate is lost and the plasma membrane follows the contours of the cell protuberances more closely (Fig. 12). MTs in the apical cortex form a network of very short, disordered bundles. The MTs extending into the secretion zone are even more pronounced than at 4 daa. They stretch deep into the long projections between the cell wall protuberances, bringing forth the complex architecture of the fully mature secretion zone (Fig. 9a, b). However, the dense band of MTs seen beneath the apical wall at 1, 2, and 4 daa is less obvious at this stage (Fig. 9b). MTs in the space between the nucleus and the apical wall are uniformly organized in a meshwork throughout the apical cytoplasm, and the concentration of MTs in the basal half of the cell has increased (Fig. 9b).

The changes in MT organization associated with development were synchronized within adjoining placental cells. Control cells where the primary antibody was omitted have only a faint background autofluorescence and occasionally show punctate staining which is clearly distinguishable from the MT labelling (Fig. 13).


1a
2a
3a
N
1b
N
2b
N
3b
4
5
6

Discussion

Plant cell morphogenesis is largely dependent on the modelling and remodelling of walls. Transfer cells acquire their unique structure through the polarized ingrowth of secondary cell wall on those face(s) of the cell presumed to be most active in solute transport (Gunning and Pate 1969).

The secondary cell wall in transfer cells of *Lilium* spp. is mainly composed of unesterified pectin and short randomly arranged strands of cellulose (Dashek et al. 1971). The development of the cell wall ingrowths starts on the day of anthesis and acquires its complex labyrinth structure 4 daa (Singh and Walles 1995). The results of the present study are summarized in a model (Fig. 15). There are two distinct arrays of MTs in the transfer cells studied, the cortical array and the central array (i.e., the MTs in the noncortical regions of the cell). The cortical array in the apical part of the cell undergoes a transition in orientation at anthesis, where the longitudinal orientation of MTs before anthesis is replaced by a transverse array. Microtubules in the basal half remain longitudinal. Reorientation of MTs occurs through dynamic instability, where old arrays break down while new MTs form with a changed orientation (Yuan et al. 1995, Hepler and Hush 1996). The transition in orientation in the apical part of the placental cells may also occur through MT depolymerization and repolymerization, which is more common in cortical MTs, rather than from the direct movement of preformed elements, as reported for some microtubular arrays (Zhang et al. 1993, Cleary et al. 1992). The longitudinal arrangement in the basal half of the cell is restricted to that portion of the cell which shares a common wall with the adjoining cells. The apical part of the cell, with walls abutting the locule, contains the transverse arrangement of MTs. Since wall ingrowths only form on the inner surface of these walls, the change in orientation of MTs in the apical half of the cell predicts the site of wall ingrowth formation.

After anthesis the transverse array in the apical part of the cell changes to a network of short, randomly arranged MTs. As the development of the secretion zone progresses, the microtubular arrangement becomes more disorganized, forming a network of interconnected strands which resembles the arrangement of cellulose microfibrils in the wall ingrowths of transfer cells (Dashek et al. 1971). It is likely that, after anthesis, MTs direct the organization of cellulose microfibrils, since MTs have an influential role in organizing the deposition of cellulose microfibrils in the walls of most plant cells (Gunning and Hardham 1982, Fosket and Morejohn 1992). Studies with tubulin microinjection indicate that MTs have the flexibility required of a changeable, three-dimensionally complex template for cellulose biosynthesis (Wymer and Lloyd 1996). MTs provide spatial cues in the cell cortex which are translated into wall patterning and thus control cell shape (Cyr 1994). Randomly organized microfibrils permit radial expansion while parallel arrays restrict expansion to an axis perpendicular to that of microtubule orientation. The distinct differ-

Fig. 1a, b. Placental cell of *L. longiflorum*, 1 dba. **a** Cortical array, the MTs are longitudinally oriented from the base of the cell to the apex. Optical projection of the cortical region through 2.4 μm of the cell. **b** Optical projection through 1.6 μm of the medial (noncortical) portion of the cell. The nucleus (*N*) occupies a major portion of the cytoplasm in the basal half of the cell. A few solitary strands of MTs can be seen in the apical cytoplasm of the cell. Bars: 5 μm

Fig. 2a, b. *L. longiflorum.* Placental cell at anthesis day. **a** Cortical MTs in the apical half of the cell make a transition from longitudinal to transverse alignment, whereas MTs in the basal half remain longitudinal. Optical projection through 2.8 μm from the outer part of the cell. **b** The nucleus (*N*) is still at the base of the cell. MTs forming the central array are much more prominent and are still seen only in the apical half of the cell. MTs appressed under the dome become evident at this stage. Optical projection through 1.6 μm of the medial portion of the cell. Bars: 5 μm

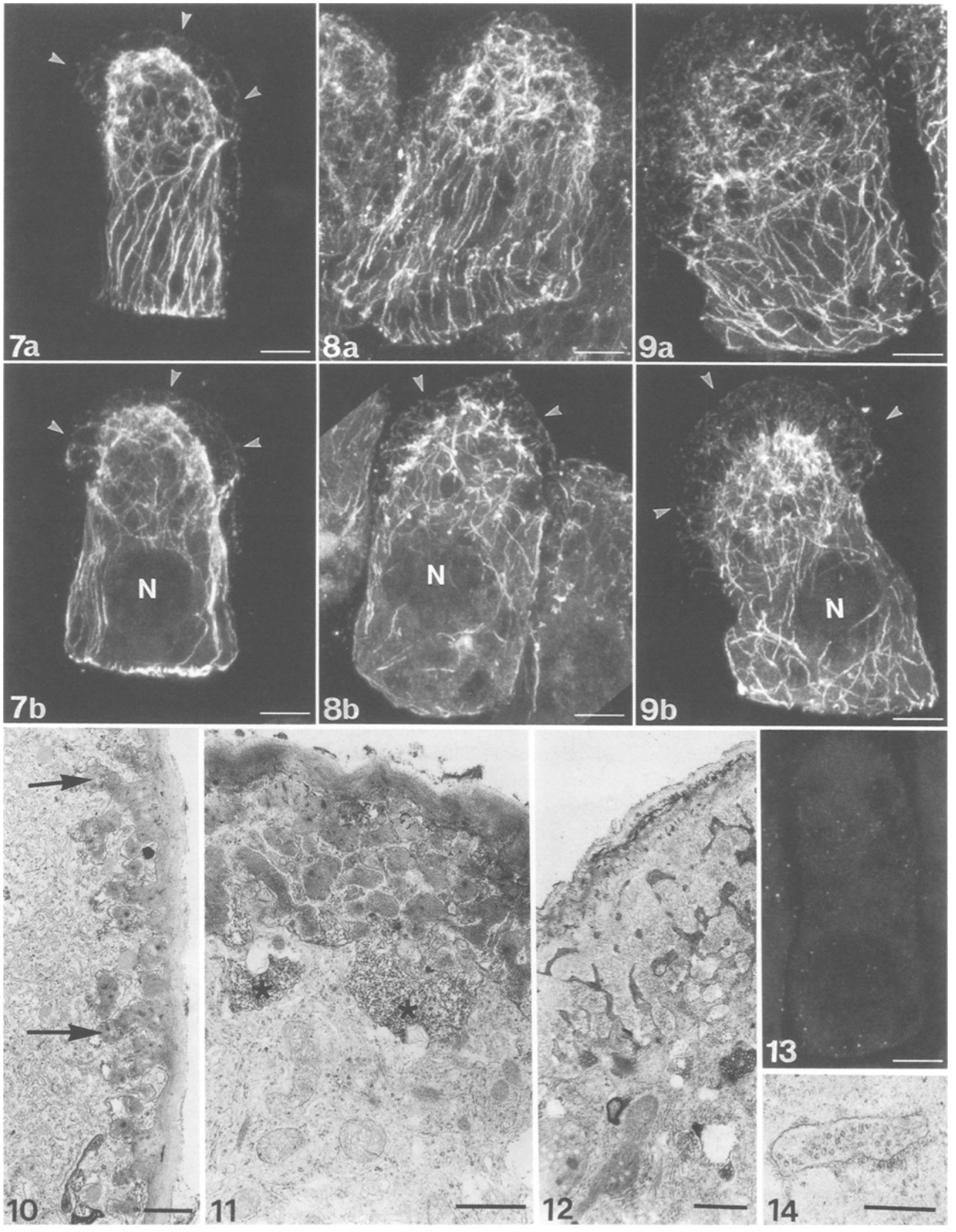
Fig. 3a, b. *L. longiflorum.* Placental cell, 1 daa. **a** Cortical array in the apical part of the cell undergoes further transition from the transverse arrangement to form a network of randomly oriented MTs. Optical projection through 2.8 μm of the cortical region of the cell. **b** The nucleus (*N*) has shifted to a more central position. A zone of MTs is appressed to the apical wall. The central array is very prominent in the apical portion of the cell. A few strands of MTs can be seen even in the basal part of the cell. Optical projection through 4.8 μm of the medial portion of the cell. Bars: 5 μm

Figs. 4–6. *L. regale.* Transmission electron micrographs of the apical cell wall of placental cells. Bars: Figs. 4 and 6, 1.5 μm; Fig. 5, 1 μm

Fig. 4. 1 dba. The cell wall is uniform in thickness. Blobs of secretions are evident on the outer surface of the cell wall

Fig. 5. At anthesis day. A few small ingrowths (arrows) project from the cell wall

Fig. 6. 1 daa. The small projections take on a more complex form, branching and anastomosing with each other to form the beginnings of a complex wall labyrinth (arrows)


7a
8a
9a
7b
N
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ence in the orientation of MTs in the two halves of the placental cell also reflects the two different kinds of expansion taking place in the cell. The basal half of the cell expands laterally where MTs are organized in longitudinal bundles, while the apical half of the cell expands more or less isodiametrically into the apical dome where MTs are in a random meshwork.

Cortical MTs in the apical part of the cell are difficult to discern in median optical section before anthesis, but they become more common at 1 daa (Fig. 15). Wall ingrowths at this stage are only slightly more developed than on anthesis day. The MTs form short, randomly arranged bundles beneath the cell wall. This organization resembles the pattern of cellulose microfibrils in the secondary cell wall. Similar parallel orientation is seen in the filiform apparatus within the egg cell of *Plumbago zeylanica* (Cass and Karas 1974). The random array of MTs appressed beneath the plasma membrane persists up to 4 daa. This coincides with secretion zone development, which is completed by 4 daa (Singh and Walles 1995).

Wall ingrowths in transfer cells protrude as tubules, increasing in depth and complexity with the addition of new wall material (Gunning and Pate 1969, Briggs 1995). The plasma membrane follows these wall protuberances, forming cytoplasmic tunnels and chambers through the whole depth of the wall thickening. In the present study MTs are seen in such furrows and tunnels at the cell apex. They are also present, at the base of the cell wall ingrowths, as short random strands, where most of the cell wall material is being deposited (Fig. 15). We propose that the MTs in the secretion zone play a role in the continued deposition of cellulose microfibrils even in the outer reaches of the cell wall. By 4 daa the secretion zone is fully mature and there is an excessive release of exudate. This exudate pushes the plasma membrane into the cell as the secretion accumulates in the wall labyrinth. However, strands of cytoplasm persist in the secretion zone and MTs are seen in these cytoplasmic strands. As the cell ages and secretion diminishes, the plasma membrane moves outward, following the contours of

Fig. 7a, b. *L. longiflorum.* Placental cell, 2 daa. **a** MTs forming the basal part of the cortical array remain in the longitudinal orientation whereas the cortical array in the apical part of the cell has a random orientation. Some MTs can be seen radiating into the secretion zone, outlining the extent of the cell wall thickness (arrowheads). Optical projection through 3.2 μm of the cortex of the cell. **b** The nucleus (*N*) remains in a central position. Cortical MTs, at the apical wall, constitute a thick band juxtaposed to the cell wall. Some MTs are also seen radiating from the central mass of the cytoplasm into the zone of cell wall projections. MTs forming the central array appear as dense, randomly oriented strands, some of which can be seen radiating from the nucleus. MTs in the basal half of the cell are still few. Optical projection of the medial portion of the cell, 4 μm deep. Note: the long strands of MTs on the left-hand side of the cell are due to an artefact brought about by infolding of the cell and thus are not a true representative of the actual organization of MTs in this part. Bars: 5 μm

Fig. 8a, b. *L. longiflorum.* Placental cell, 4 daa. **a** MTs in the cortex of the apical part of the cell are organized in a random meshwork of short strands. MTs in the basal part of the cortex remain longitudinal in orientation. Optical projection through 2.5 μm of the cortex of the cell. **b** The nucleus (*N*) stays in the slightly central position. A thick band of MTs is still seen appressed to the cell wall, beneath the secretion zone. Larger number of MTs are seen in the protoplasmic projections between the cell wall ingrowths (arrowheads). A profusion of randomly oriented MTs occurs in the apical and lateral portions of the cell. A small number of MT bundles are seen in the basal portion of the cell. Optical projection of medial portion of the cell, 6 μm thick. Bars: 5 μm

Fig. 9a, b. *L. longiflorum.* Placental cell, 8 daa. **a** Cortical MTs in the basal part of the cell are still longitudinal and in the apical half they constitute a random meshwork, although MTs in the inner reaches of the secretion zone are more profuse. Optical projection through 3.5 μm from the plasma membrane. **b** An extremely dense profusion of criss-crossing MTs is in the apical portion of the cell. The band of MTs appressed to the cell is not as prominent at this stage. A profusion of MTs (arrowheads) radiates into the secretion zone showing the complex extent of the cell wall ingrowth. MTs have also increased in density in the basal half of the cell. Optical projection of the medial portion of the cell, 6 μm deep. Bars: 5 μm

Figs. 10–12. *L. regale.* Transmission electron micrographs of the apical cell wall of placental cells. Bars: 1 μm

Fig. 10. 2 daa. The cell wall is more than twice the thickness of the original wall. Wall protuberances (arrows) have a complex architecture, but the plasma membrane still follows the contours of the cell wall projections

Fig. 11. 4 daa. The cell wall has achieved its maximum thickness and the elaborate secretion zone is fully mature. The plasma membrane is pushed inwards due to the accumulation of exudate (asterisks) under the secretion zone

Fig. 12. 8 daa. The secretion zone remains unchanged after 4 daa, but there is no more accumulation of exudate so the plasma membrane once again follows the contours of the cell wall ingrowths

Fig. 13. *L. longiflorum.* Control sample from 8 daa, where the primary antibody was omitted, shows a faint autofluorescence and a weak punctate staining, clearly distinguishable from the MT labelling. Bar: 5 μm

Fig. 14. *L. regale.* Electron micrograph of cross section through an MT bundle in the inner reaches of the cell wall projections at 4 daa. Bar: 1.5 μm

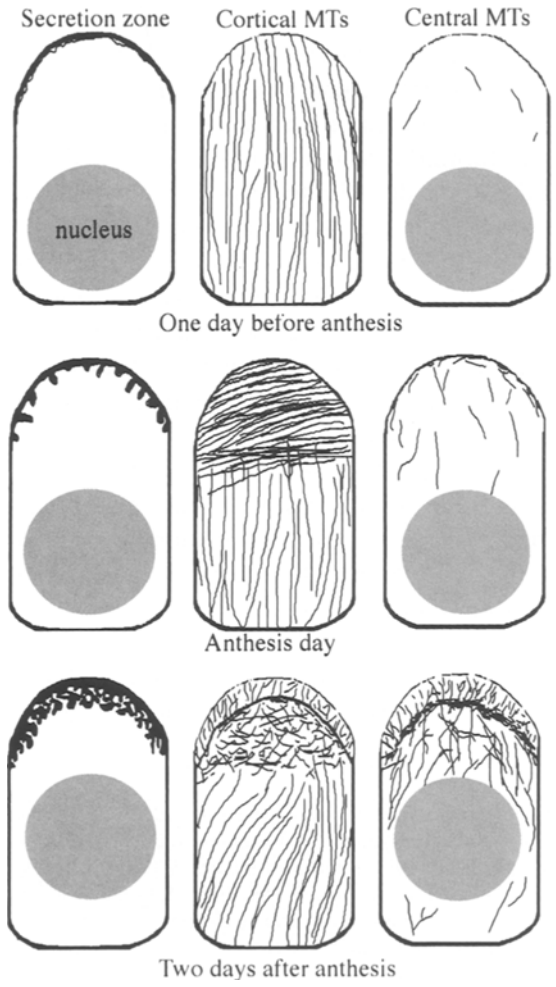

Fig. 15. This model of the placental transfer cells depicts the development of the secretion zone and accompanying changes in the microtubule arrays at three crucial stages of development

the cell wall once again, and MTs are more densely integrated in the wall protuberances. The presence of MTs at this late stage implies that they may have another function since there is no further addition to the cell wall.

The second microtubule array is in the noncortical region of the cell and is referred to as the central array (Fig. 15). Similar arrays are reported in other cells with wall ingrowths, including synergids (Huang and Russel 1994, Ye et al. 1996) and transfer cells of *V. faba* cotyledons (Bulbert et al. 1998). This array is distinct from the radial array more commonly found in various kinds of cells. The density of microtubule bundles beneath the apical wall increases parallel with the increase in development of the secretion zone from 1 to 4 daa, suggesting that these MTs are involved in the formation of the secretion zone at this stage. Bulbert et al. (1998) propose that MTs beneath the apical wall in *V. faba* transfer cells provide a cytoskeletal framework to establish a polarized distribution of dictyosomes, mitochondria, and endoplasmic reticulum. They could also play a role in stabilizing these cytoplasmic components associated with the wall ingrowths. Evidence of MTs associated with cell organelles in the vicinity of wall ingrowths was reported in synergids (Huang and Russell 1994). Although there is evidence that actin microfilaments direct the transport of vesicles in many plant systems (Williamson 1993), microtubules may also be involved (Li et al. 1997). Emons et al. (1992) propose that MTs direct Golgi vesicles to insertion points on the plasma membrane. It is possible that MTs in the central array are involved in transport of vesicles to the secretion zone.

Bulbert et al. (1998) observed that in transfer cells of *V. faba* embryo cotyledons, cortical MTs were present at all the faces of the cell before the appearance of wall ingrowths. However, in cells with well developed wall ingrowths, MTs were localized almost exclusively in the cortical regions adjacent to the wall ingrowths. They concluded that the ingrowth probably results from the directed delivery of vesicles containing precursor material to the cell wall, rather than from deposition of additional layers of organized cellulose microfibrils. A similar model was also proposed by Emons and co-workers (Emons et al. 1992, Emons and Kieft 1994). In placental cells of *L. longiflorum*, MTs in the cortex are clearly present throughout development and show a series of unique reorientations during the process of cell maturation. More significantly, these transitions in orientation parallel the buildup of wall ingrowths. The underlying reason for the differences in our observations and those of Bulbert et al. (1998) may be that placental cells in *Lilium* spp. have a predominantly secretory role while transfer cells in developing *V. faba* cotyledons function in the uptake of sugars (Bulbert et al. 1998). Our interpretation is that in placental cells of *Lilium* spp., the cortical MTs at the secretion zone predict the site of deposition of secondary-cell-wall material and also act as templates for the orientation of cellulose microfibrils. This conclusion is based on the similarity in patterning of the microfibrils and the underlying MTs. In all other systems with secondary-cell-wall growth, MTs delineate the site of secondary-wall deposition and also orchestrate the pattern of cellu-

lose microfibrils. In conclusion, our observations on Lilium transfer cells are in accord with the view that cortical MTs in association with cellulose-synthesizing complex in the plasma membrane comprise a unique mechanism for the structured laying down of microfibrils, thus adding transfer cells to the list of cell types with secondary-cell-wall growth, in which MTs have been found to align wall microfibrils.

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