Protoplasma (1996) 193: 181–190

PROTOPLASMA

The vacuolar-tubular continuum in living trichomes of chickpea (*Cicer arietinum*) provides a rapid means of solute delivery from base to tip

M. D. Lazzaro[1, *], and **W. W. Thomson**[2]

[1] Department of Botany, Stockholm University, Stockholm and [2] Department of Botany and Plant Science, University of California, Riverside, California

Received December 1, 1995
Accepted February 14, 1996

Dedicated to Prof. Dr. Dr. h.c. Eberhard Schnepf on the occasion of his retirement

Summary. A vacuolar continuum exists from base to tip in the secretory trichomes of chickpea (*Cicer arietinum*). This continuum is seen in living trichomes which have been labeled with Lucifer yellow CH and examined with confocal microscopy. It encompasses the large vacuole of the lower stalk cell, the vacuoles and tubules of the central stalk cell, the thin tubules of the upper stalk cell, and the tubules and vacuoles of the secretory head cells. The vacuolar-tubular system is structurally distinct within each cell, forming a gradient of large vacuoles in the lower stalk cell, thick tubules in the central stalk cell, and thin anastamozing tubules in the upper stalk cell. This membrane system appears to be continuous between trichome cells, as thin tubules emanate from plasmodesmata between stalk cells and between the upper stalk and lower head cell. In the upper stalk cell, the thin tubules of this continuum are streaming up and down the long axis of the cell at 0.67 μm/s. The larger vacuolar-tubular system in the central and lower stalk cells is also slowly moving, with apparent peristalsis occurring in the central cell. The vacuolar-tubular system of the secretory head cells is completely labeled with Lucifer yellow when the dye has only partly diffused up the long walls of the trichome, indicating that the streaming tubular system delivers solute through the stalk cells to the secretory head cells faster than diffusion through the trichome walls. In the lower head cells, tubules emanate from the plasmodesmata connecting to the upper stalk cell, and these tubules are continuous with the head cell vacuoles. In addition, another layer of thin tubules forms along the edges of the secretory head cells, at the site of exocytotic secretion. We propose that the continuous vacuolar-tubular system in these trichomes functions to rapidly deliver solute from the base of the trichome to the secretory head cells. This system provides a pathway for the transport of secretory material.

* Correspondence and reprints: Department of Botany, Stockholm University, S-106 91 Stockholm, Sweden.
E-mail: lazzaro@botan-su-se

Keywords: *Cicer arietinum*; Confocal microscopy; Lucifer yellow CH; Plasmodesmata; Trichomes; Vacuoles.

Introduction

The trichomes of chickpea (*Cicer arietinum*) have a basal cell, long vacuolate stalk cells, and a terminal cluster of dense secretory head cells (Schnepf 1965, Lazzaro and Thomson 1989). Schnepf first described the ultrastructure of these secreting trichomes, particularly the head cells, in 1965 when electron microscopy was emerging as a premier tool in plant cell biology. Twenty-five years later we published further ultrastructural details of the head cells as well as the organizational features of the stalk and basal cells (Lazzaro and Thomson 1989).

The secretions from these trichomes appear to protect the plants from insect predation (Srivastava and Srivastava 1989). Recently, it has been shown that the trichomes secrete hydrochloric acid (pH 0.52), malic acid, and calcium (Lauter and Munns 1986, Lazzaro and Thomson 1995). Schnepf (1965) first observed that the head cells had numerous mitochondria and made the quite reasonable suggestion that the secretion process is highly energy dependent. However, the head and stalk cells do not have developed chloroplasts (Schnepf 1965, Lazzaro and Thomson 1989) and thus carbon substrate essential to fuel the energy cost of secretion must flow through the stalk cells to the head cells (Lazzaro and Thomson 1989, 1992a, b).

Using non-toxic levels of lanthanum, an apoplastic marker (Revel and Karnovsky 1967, Thomson et al. 1973), we found that this electron dense probe was taken into the stalk cells and sequestered into the vacuolar system through pinocytosis (Lazzaro and Thomson 1992a). The fluorescent dye Lucifer yellow CH is also non-toxic and will not cross the plasma membrane of intact cells (Stewart 1978), so is an ideal apoplastic marker. In the present study we observed that exogenously applied Lucifer yellow is also taken up pinocytotically and sequestered in the vacuolar system of the stalk cells. The dye then moves through and labels the entire vacuolar complex of the trichome cells, including the secretory head cells. Using fluorescently labeled living cells, we find that the vacuolar complex, or more accurately the vacuolar-tubular system, is continuous from cell to cell within these secretory trichomes. The ultrastructural definition of this continuum is lost during conventional fixation.

Material and methods

Leaves of *Cicer arietinum* L. cv. UC5 were excised and dipped into distilled water to rinse off secretion droplets. The petiole was then cut again, the cut end was placed in a microfuge tube containing 8.5 mM Lucifer yellow CH dipotassium salt (Cole et al. 1991) in distilled water, and these tubes were then placed in a humid chamber to hasten the reformation of secretion droplets. In some experiments, leaves were first placed in a solution of 5.0 mM probenecid (pH 7.0) for 2 h and then transferred to 8.5 mM Lucifer yellow CH (Oparka et al. 1991). Dye uptake proceeded for 6 to 24 h. Fresh sections of leaflets were then cut for microscopy. Trichome cells were examined with the Bio-Rad MRC 600 laser scanning confocal microscope using the CM program. The "BHS" filter block was used, with an excitor filter which selects a 10 nm wide laser line at 488 nm, a dichroic reflector (DR 510 LP) and a 515 nm barrier emission filter. The ×60 oil immersion objective (Nikon) was used (numerical aperture 1.4), so the theoretical resolution limit of the confocal images is 174 nm. Still images were enhanced with Kalman averaging, while time lapsed images were collected with the direct setting on slow speed and written automatically to the computer hard drive.

Subsequent image processing was completed using "NIH Image" on a Macintosh computer. We measured the cross sectional diameter of the vacuolar-tubular system by measuring tubules which intersected transverse axes drawn at 20 μm intervals on longitudinal sections of the stalk cells. The cell images were calibrated with images of a stage micrometer recorded at the same magnification.

To measure tubule motility, images were collected with the "Direct" setting on normal speed, resulting in a laser scan of the cell every 1.06 s. These images were recorded for analysis by videotaping the image monitor of the confocal microscope. The video tapes were played back and a ruler was used to measure organelle movement across a flat video monitor, calibrated with a stage micrometer. For motility measurements, stalk cells were scanned and recorded for 2–3 min each. Tubules were judged to be moving directionally when their movement was consistently up or down for at least 4 consecutive scans (average of 13 consecutive scans), and only these tubules are included in the motility data. Probenecid treated material was never used to measure streaming rates.

Results

Lucifer yellow labels the entire vacuolar system of the secretory trichomes after being taken up by pinocytosis in the central stalk cell (Fig. 1). Since the only detectable autofluorescence within the trichomes is from the small chloroplasts in the basal cell (Fig. 2 a, b), all fluorescent labeling in treated material results from Lucifer yellow.

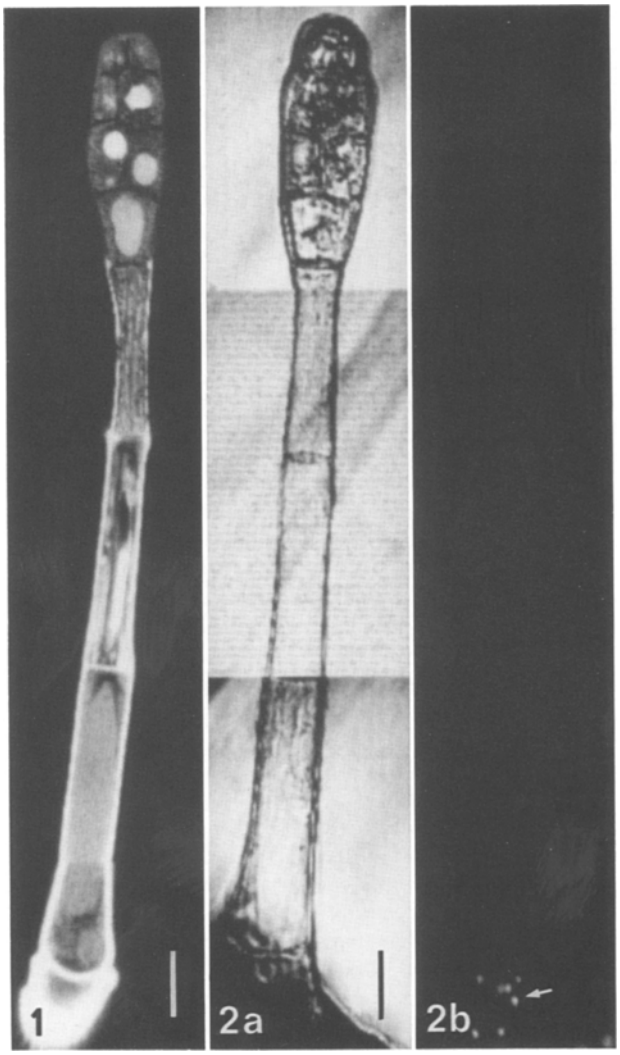


Fig. 1. Lucifer yellow labeled the entire vacuolar-tubular system of the trichome, including the three stalk cells and the head cells

Fig. 2. In control trichomes treated with distilled water, only the chloroplasts in the basal cell were visible with fluorescent microscopy (arrow). Stalk and head cells had no autofluorescence. Nomarski DIC (**a**) and confocal fluorescence (**b**) pair. Bars: 25 μm

The vacuolar system is structurally distinct between each stalk cell. The lower stalk cell has large vacuoles (Fig. 3 a), which occasionally branch near the transverse wall (Fig. 3 b, c). The central stalk cell contains a dynamic vacuolar system, ranging from large vacuoles (Fig. 4 a) to tubules (Fig. 4 b, c). This dynamic range is seen within single cells as vacuoles extend into tubules which run the entire length of the stalk cell (Fig. 4 a–c). The tubules also contain distinct, swollen regions (Fig. 4 c) and form complex networks (Fig. 4 b). The upper stalk cell contains the thinnest tubules (Fig. 5 a–e). These tubules traverse the length of the stalk cell in long parallel arrays and also branch into complex patterns (Fig. 5 a–e). When examined in serial section, it is clear that the thin tubules are present just beneath the plasma membrane (Fig. 5 a) and form a continuous network deep into the stalk cell (Fig. 5 b–e).

The vacuolar-tubular system is continuous between stalk cells. Thin tubular branches, 200–500 nm in width, extend from the larger vacuolar-tubular system and lead to the transverse walls between the upper stalk and head cells, and distinct fluorescent bands across the transverse wall connect these tubules between cells (Fig. 6 a). In addition, the tubules in the head cells lead directly into small vacuoles (Fig. 6 a). Thin tubules also lead to the transverse walls between the central and upper stalk cells (Fig. 6 b) and between the lower and central stalk cells (Fig. 6 c). These tubules lead to the same area on either side of the transverse wall, in likely connection across the wall. We conclude that the bands across the wall are concentrated groups of plasmodesmata traversed by fluorescently labeled tubular membrane strands that connect the tubules in the upper stalk and lower head cells, and that a similar connection exists across the transverse walls between stalk cells.

A network of thin tubules (680 nm average diameter) is present along the edges of the head cells (Fig. 7 a, b). These tubules are at the site of secretion from the head cells, and may have a direct role in the secretion process. In serial section, it is clear that further into the cells, these tubules connect directly to small vacuoles (Fig. 7 c, d). The entire vacuolar-tubular system of the head cells and upper stalk cell is labeled with Lucifer yellow when the dye has diffused only partly up the long trichome walls (Fig. 8), indicating that the vacuolar-tubular system delivers the dye to the secretory tip of the trichomes faster than diffusion through the wall. The peripheral tubules in Fig. 7 are from the same trichome shown in Fig. 8, so the Lucifer yellow in these peripheral tubules must have come from the vacuolar-tubular system, and not from the cell walls. There is a clear quantitative difference between the vacuolar system in the three stalk cells. We measured the cross sectional diameter of the vacuolar-tubular system at 20.0 μm intervals along the three cells and found that in the lower cell, the vacuolar-tubular system ranged from large vacuoles which filled the entire cell diameter (up to 16.8 μm), to thinner branches (0.9–2.0 μm in diameter) at the end of the cell. The central stalk cell also had a diverse vacuolar-tubular system, but overall the tubules were thinner than in the lower cell, since 96.5% of the tubules were between 0.4 and 6.0 μm in diameter. Quantitatively, the upper stalk cell was unique. All of the tubules in the upper cell were between 0.2 and 2.8 μm in diameter, and 85.7% of them were less than 1.1 μm in diameter (Table 1).

The vacuolar-tubular system moved within the stalk cells. In the lower and central stalk cells, the larger vacuoles slowly moved in and out of the focal plane of the confocal microscope (not shown). In addition, slow directional movement (0.49 ± 0.18 μm/s) was seen in the central stalk cell. Swollen regions in the central stalk cell emerged into the plane of focus and then moved up towards the trichome tip in apparent peristalsis (Fig. 9). Within the upper stalk cell, the thinner tubules moved faster and more consistently than in the central cell (Fig. 10). These tubules move both up and down the stalk cell at 0.67 μm/s (Table 2).

Discussion

The membrane impermeant fluorescent dye, Lucifer yellow CH, has proven useful as a general apoplastic marker (Oparka and Prior 1988), but it has also been shown to enter plant cells and become sequestered within the vacuole (for reviews, see Robinson and Hillmer 1990, 1991; Oparka 1991). We have assumed that uptake and transport of the dye to the stalk cell vacuoles of the chickpea trichomes is by fluid phase endocytosis, based on our earlier observations that this mechanism occurs with the uptake of lanthanum observed in the electron microscope (Lazzaro and Thomson 1992a). There is the possibility that Lucifer yellow crosses the plasma membrane through probenecid sensitive anion transport (Cole et al. 1991; Oparka 1991, 1993; Wright et al. 1992). We tested this possibility by treating trichomes with 5.0 mM

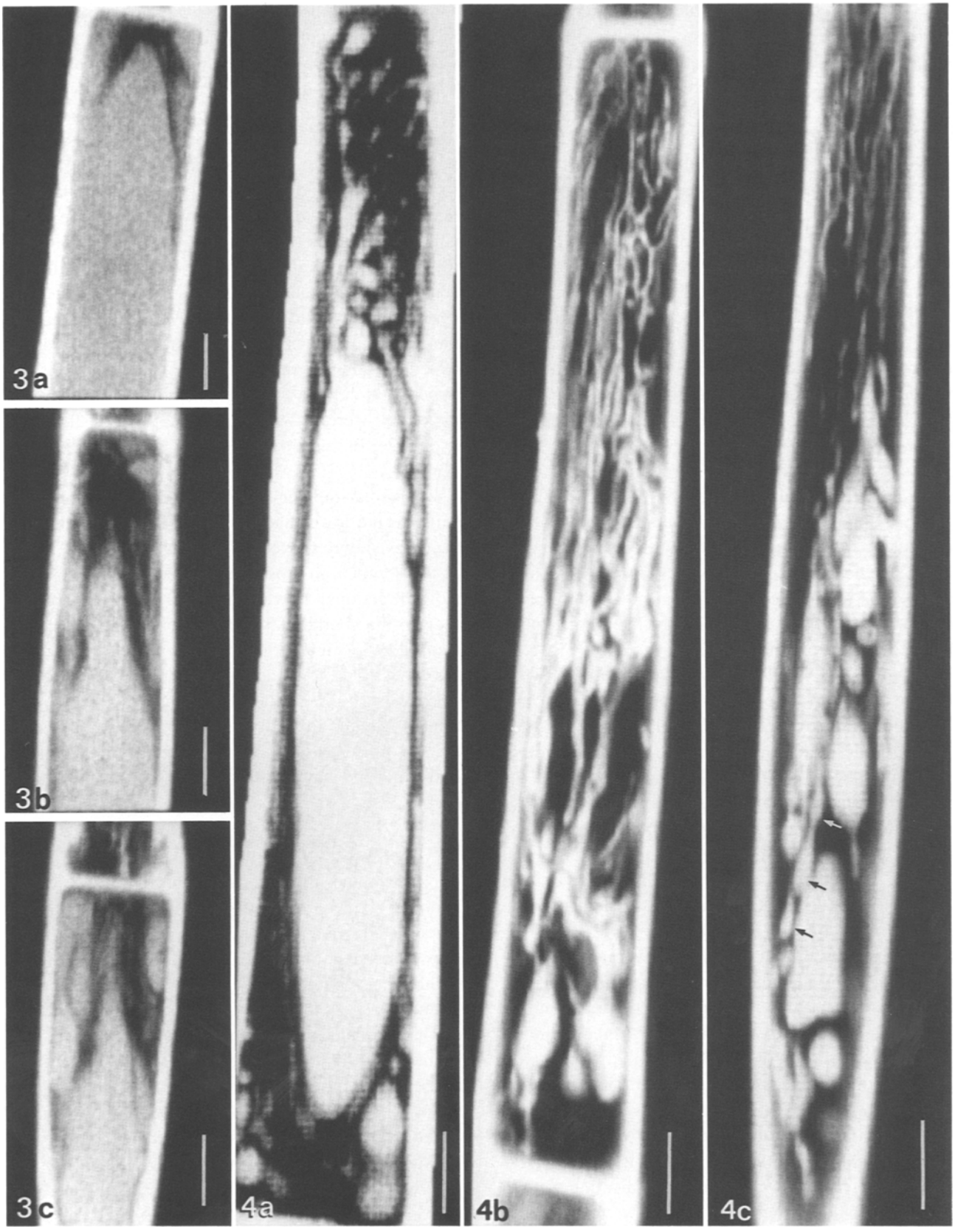

3a
3b
3c
4a
4b
4c

Table 1. The cross sectional diameter of the vacuolar-tubule system was measured in the lower, central, and upper stalk cells

Size category (μm)	Distribution (%)		
	lower	central	upper
0.0–1.0	4.5	25.7	85.7
1.1–2.0	28.1	49.6	13.8
2.1–3.0	14.6	11.9	0.5
3.1–4.0	6.7	4.8	0.0
4.1–5.0	7.9	2.3	0.0
5.1–6.0	3.4	2.2	0.0
6.1–7.0	5.6	0.8	0.0
7.1–8.0	2.2	0.2	0.0
8.1–9.0	6.7	0.5	0.0
9.1–10.0	0.0	0.2	0.0
10.1–11.0	3.4	0.0	0.0
11.1–12.0	1.1	0.5	0.0
12.1–13.0	5.6	0.3	0.0
13.1–14.0	1.1	0.5	0.0
14.1–15.0	4.5	0.3	0.0
15.1–16.0	0.0	0.3	0.0
16.1–17.0	4.5	0.0	0.0

Measurements were taken of all vacuolar-tubules that crossed lines drawn at 20 μm intervals perpendicular to the long axis of the stalk cells. In the lower stalk cell, tubule diameters ranged from filling the main body of the cell (large vacuoles up to 16.8 μm in diameter) to thin branches (0.9 μm) at the cell ends. Within the central stalk cell, the vacuolar system was primarily composed of tubules less than 6.1 μm in diameter (96.5%), although vacuoles up to 15.7 μm in diameter were occasionally present. In the upper stalk cell, all the vacuolar-tubules were between 0.2 and 2.8 μm in diameter. The average tubule diameter was 5.4 μm for the lower stalk cell, 1.9 μm for the central stalk cell, and 0.7 μm for the upper stalk cell. 1,118 tubule cross sections were measured in 5 lower, 8 central, and 7 upper stalk cells

Table 2. Within the upper stalk cell, the thin vacuolar-tubules were streaming both up towards the tip of the trichome and down towards the base

Rate of movement (μm/s)	Number of moving tubules	Direction
0.67 ± 0.27	41	up
0.67 ± 0.27	35	down

Streaming was observed in 13 recorded cells. Not all tubules were moving within cells, and only those that were moving in one direction for at least 4.3 s were used in the rate calculations. The average duration of unidirectional movement was 13.8 s, with some tubules moving up to 48.8 s in one direction. Rates are means ± standard deviations

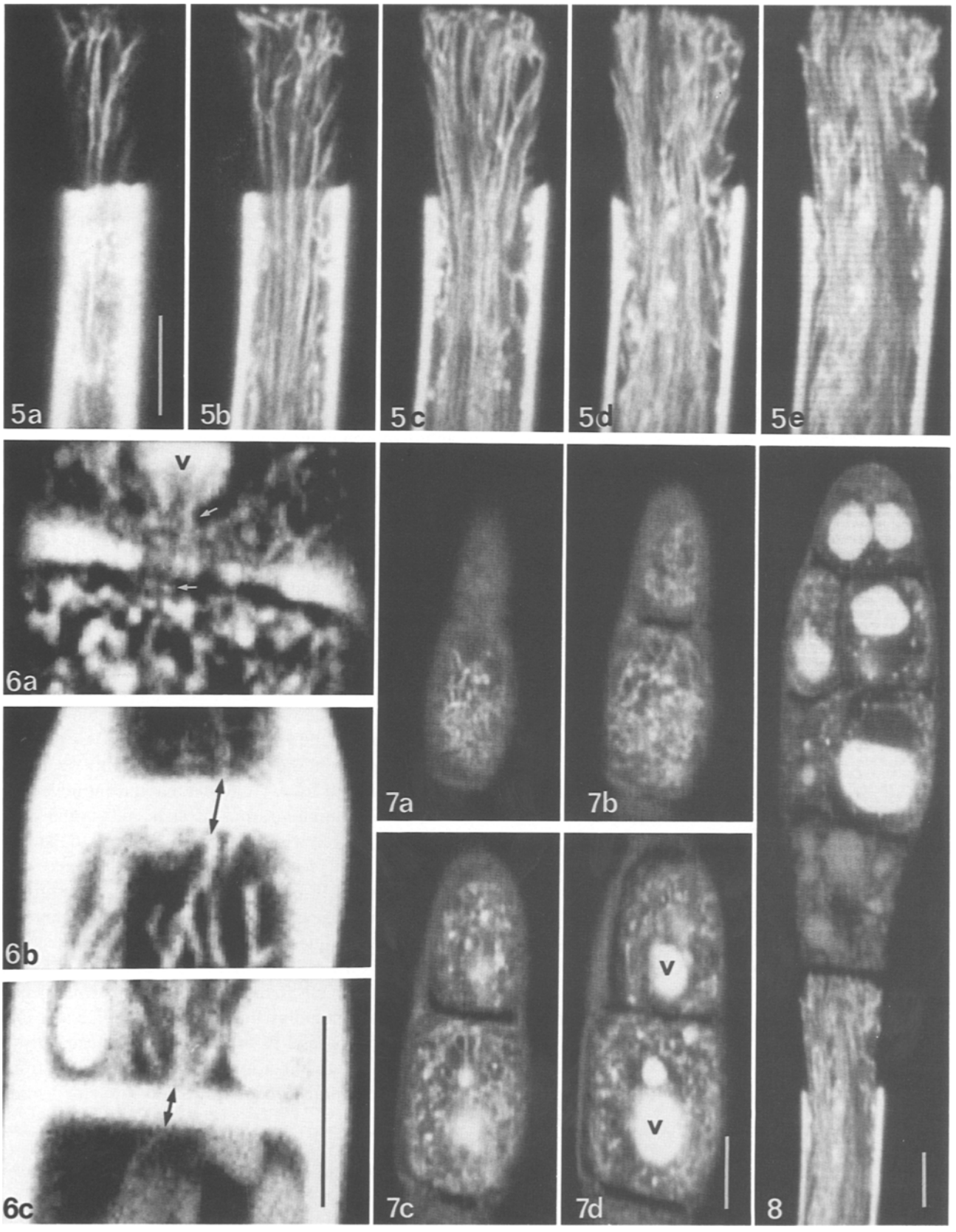
probenecid in some experiments (Oparka et al. 1991) and found no difference in the localization of dye within the vacuolar system. In fact, all figures except 4 a, 9, and 10 are from probenecid treated material.

We found that Lucifer yellow accumulated in the vacuolar complex of the stalk cells and then diffused throughout the entire vacuolar system of stalk and head cells. This vacuolar-tubular system was structurally and dimensionally distinct within each stalk cell, and a morphological gradient was apparent. Lower stalk cells contain large vacuoles with tubular extensions, central stalk cells have a vacuolar complex consisting mainly of elongate tubules, and upper stalk cells have anastomozing tubular strands. This vacuolar-tubular system streams slowly up and down the long axis of the stalk cells with the thin tubules in the upper stalk cell moving faster than the vacuolar-tubular system in the lower stalk cells. The vacuolar-tubular system appears to be continuous between cells by way of strands extending to, through, and from plasmodesmata in the walls between trichome cells. This continuum extends into the head cells via tubules emanating from the plasmodesmata into vacuoles within the head cells. We found that the entire vacuolar-tubular continuum from the stalk cells to the head cells was labeled with Lucifer yellow prior to the diffusion of the dye through the walls of the stalk cells and into the walls of the head cells. From these observations we suggest that solute is first taken up pinocytotically and sequestered in vacuoles of the stalk cells, and then diffuses within the streaming continuum from the stalk cells to the head cells. Thus, this vacuolar-tubular continuum functions to rapidly deliver solute from the base of the trichomes to the head cells.

There are several other implications to be drawn from these observations. If the solutes are maintained with-

Fig. 3. In the lower stalk cell, the vacuolar-tubular system ranged from a central vacuole filling the whole cell diameter (**a**), to thinner tubules which branched from the central vacuole (**b** and **c**). These tubules were predominantly at the upper transverse wall

Fig. 4. The vacuolar-tubular system within the central stalk cell included large vacuoles which extended into thick tubules running the length of the cell (**a**). Thin, anastomozing tubules also ran the length of the central stalk cell (**b**). In addition, the tubules formed swollen regions (arrows) along their length (**c**). Bars: 10 μm. The images in Figs. 3 and 4 are from 6 different cells


5a
5b
5c
5d
5e
v
6a
6b
6c
7a
7b
7c
v
v
7d
8

in this vacuolar-tubular continuum through the plasmodesmata between cells, this would indicate that the central membranous component of these plasmodesmata is continuous with the tonoplast membrane that delimits the boundary of the vacuolar-tubular extensions. We note that we did not detect the extent and form of the vacuolar-tubular complex with probable continuities to and through the plasmodesmata in our previous ultrastructural studies (Lazzaro and Thomson 1989, 1992a, b). This was probably due to the two dimensional images produced by TEM examinations of thin sections as opposed to the three dimensional view of cell organization provided by confocal microscopy. It is very important that in the present study, *living* cells were examined. The complexity of the vacuolar continuum is probably disrupted during conventional fixation for electron microscopy (see O'Brien et al. 1973, Mersey and McCully 1978, Dong et al. 1994, for discussion of the fidelity of fixation procedures). In our previous ultrastructural studies, we interpreted the membranous, bubble-like extensions from the axial component of the plasmodesmata to be extensions of the endoplasmic reticulum (Lazzaro and Thomson 1989). This was and still is the most common interpretation in the literature (Robards and Lucas 1990, Lucas et al. 1993). However, we suggest that these blister-like bubbles extending from the axial component of the plasmodesmata are components of the vacuolar-tubular continuum and that they assume a vesiculate appearance with the disruption of the thinner elements of the continuum during fixation. It may be imprudent to identify such membrane extensions from plasmodesmata as ER in all instances, particularly in mature, vacuolate cells.

Our view of the vacuolar-tubular continuum as a path for solute diffusion from cell to cell would infer a passageway through the plasmodesmata via a channel in the center of the axial component. Current views of plasmodesmatal structure and function tend to minimize the possibility of trafficking of ions, water or small molecular species via a channel through the center of the axial component. These views are based primarily on ultrastructural observations (for reviews, see Robards and Lucas 1990, Lucas et al. 1993) and theoretical considerations (Overall et al. 1982). However, the presence or absence of an axial channel has not been definitively established with ultrastructural studies, nor precluded on deductive grounds (Gunning and Overall 1983). Even rapidly frozen, freeze substituted material (Ding et al. 1992) cannot be assumed to be free of preparation induced alterations. These alterations may be subtle and small, but significant considering the small dimensions and structural parameters of plasmodesmatal organization.

The streaming of the vacuolar-tubular continuum in the stalk cells transports Lucifer yellow to the trichome tip faster than diffusion alone through the walls. We propose that this streaming may be directed and controlled by the cytoskeleton within stalk cells. We have observed that vesicles are aligned in long arrays parallel to microtubules oriented from base to tip in the stalk cells (Lazzaro and Thomson 1989). These vesicles were observed in conventionally fixed material, and represent the vacuolar-tubular continu-

Fig. 5. Thin tubules were always present throughout the upper stalk cell, forming either long parallel arrays or branching into complex patterns. Images are 0.5 μm thick optical serial sections at 1.0 μm intervals, beginning at the plasma membrane (**a**) and continuing into the stalk cell (**b–e**). After only three hours of incubation in dye solution, tubules at the top of the cell contain Lucifer yellow while the dye has only partially diffused up the lateral cell walls

Fig. 6. Thin tubules of the vacuolar-tubular system connect across the transverse wall between the upper stalk cell (**a**) and the head cells (arrows). Distinct fluorescent bands are seen across this transverse wall. Within the head cells, these tubules lead directly into the small vacuole (*v*). Tubules from the central stalk and upper stalk cells (**b**) lead to the same area on either side of the transverse wall between the cells (double headed arrow). In addition, thin tubules in the lower stalk and central stalk cells (**c**) lead to the same area at the transverse wall between these cells (double headed arrow)

Fig. 7. Along the periphery of the head cells, where secretion takes place, a network of thin tubules is loaded with Lucifer yellow. The average diameter of these tubules was 680 ± 150 nm (n = 85 tubules from 4 cells). In successive 0.5 μm thick optical serial sections at 1.0 μm intervals, the dye is present within tubules at the periphery of the head cells (**a** and **b**), while further into the cells the same tubules are connected with small vacuoles (*v*) (**c** and **d**). The peripheral tubules are labeled with dye that has come from the vacuolar-tubular system, since the cell walls are not labeled

Fig. 8. Lucifer yellow has diffused partly up the long walls of the upper stalk cell, while the entire vacuolar-tubular system of the head cells is labeled, indicating that the dye has moved through the vacuolar-tubular system of the stalk into the head cells. This is the same trichome at higher magnification as in Figs. 5 a–e and 7 a–d. Bars: 10 μm

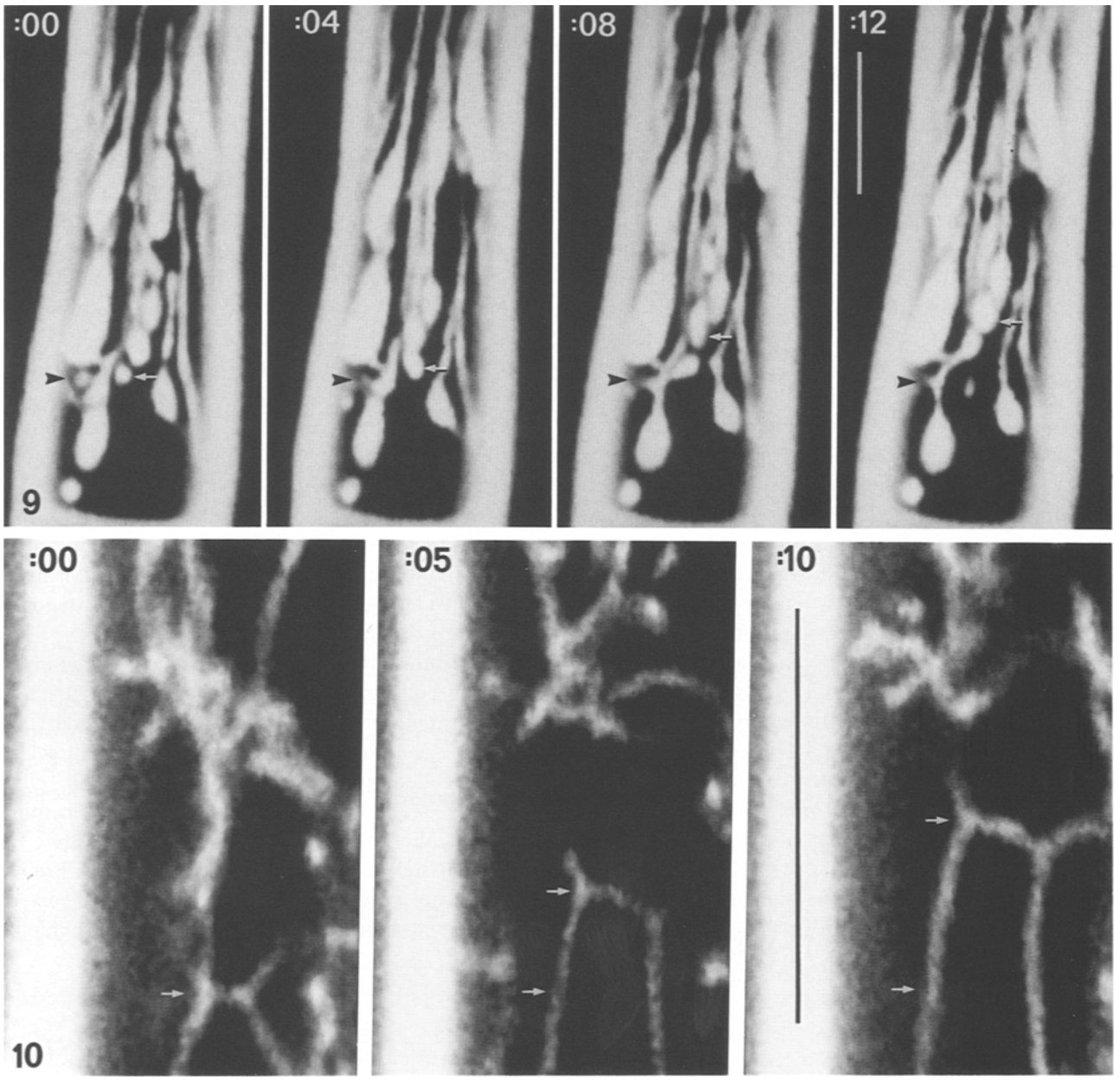


Fig. 9. Time lapsed series of images of the central stalk cell where the vacuolar-tubular system is slowly moving. Swollen regions form in the plane of focus and then move up towards the top of the cell. The arrowhead is in the exact same place throughout the time series and marks the point where thin branching tubules form a swollen emerge. A single swollen region is marked with an arrow as it moves up towards the trichome tip over time in apparent peristalsis. It is moving up at 0.35 μm/s. Images are at 4 s intervals. Bar: 10 μm

Fig. 10. Series of time lapsed images from the upper stalk cell demonstrating that the thin tubules move directionally within the cell. In this example, the branch point in the tubule is moving up towards the tip of the trichome at 0.45 μm/s. The lower arrow is in the exact same location in each frame and marks the initial position of the branch. The upper arrows identify the location of the branch point at 5 and 10 s. Bar: 10 μm

um seen in the present study (see also O'Brien et al. 1973). In staminal hairs of *Tradescantia blossfeldiana*, both microtubules and microfilaments have a role in cytoplasmic streaming (Salitz and Schmitz 1989), and actin microfilaments have recently been found associated with plasmodesmata between plant cells (White et al. 1994). The thin tubules in the upper stalk cell are streaming at 0.67 μm/s. This rate is much slower than myosin driven motility along microfilaments (26 μm/s; Kohno and Shimmen 1988), and is

also slower than the actin-myosin based movement (Liebe and Quader 1994) of the ER in epidermal cells (up to 4 μm/s; Knebel et al. 1990). The rate is similar to kinesin driven motility along microtubules (0.6 μm/s; Vale et al. 1985), and kinesin has been identified in higher plants (Tiezzi et al. 1992, Cai et al. 1993, Mitsui et al. 1993, Liu et al. 1994). The vacuolar-tubular continuum in these trichomes may be an intriguing model system for investigating cytoskeletal function in a secretory system.

Within the central stalk cell, swollen regions progress through the tubular system in apparent peristalsis. This type of peristaltic movement also occurs within the tubule-vacuole continuum in fungal hyphae to transfer solute between vacuole clusters (Shepherd et al. 1993a). In chickpea trichomes, pinocytosis of solute occurs in the central stalk cell (Lazzaro and Thomson 1992a), and the observed peristaltic movements in the cell may transport this solute towards the trichome tip for secretion. Peristaltic movements were not seen in the upper stalk cell, where thinner tubules (less than 2.8 μm in diameter) streamed faster than the thicker tubules (less than 6.1 μm) in the central cell. In the upper cell, streaming was measured as tubule tips moved up or down for at least 4 s before moving out of the focal plane, and tubular diameter did not change during these movements.

A network of tubules and vacuoles, the "peripheral caniculate system" (Mersey and McCully 1978, McCully and Canny 1985), as well as an extensive, peripheral system of ER, the "peripheral reticulum" (Quader et al. 1989, Knebel et al. 1990) are present in plant cells. Although their role in cell-to-cell transport has not been explored, these systems may be related to the tubular-vacuole continuum in chickpea trichomes. A dynamic continuum of tubules and vacuoles has been identified in fungal hyphae (Shepherd et al. 1993a), and tubules in this system pass through the dolipore in the wall between the terminal and penultimate tip cells (Shepherd et al. 1993b). To what extent the diffusion of vacuolar solution from cell to cell occurs in plant cells is conjectural at this time. However, in chickpea the diffusion of vacuolar solution from base to tip in the trichomes may have a direct role in secretion. These trichomes secrete hydrochloric acid, malic acid, and calcium (Lauter and Munns 1986, Lazzaro and Thomson 1995), all these compounds are actively sequestered in vacuoles in plant cells (Martinoia 1992). The vacuolar-tubular continuum in the trichomes may deliver these compounds to the site of secretion in the head cells. Calcium and chloride ions must be transported to the trichome tip for secretion, and since the stalk and head cells lack chloroplasts (Lazzaro and Thomson 1989), carbon substrate must also be transported from the basal cells for the secretion of malate. In fact, calcium has been localized in the vacuolar-tubular continuum in stalk cells and in head cells, and is secreted via exocytosis in the head cells (Lazzaro and Thomson 1992b). Since there are no plasmodesmata connecting the basal cell of the trichome to other cells of the leaf (Lazzaro and Thomson 1989), the vacuolar-tubular continuum is limited to the trichomes. Malate synthesis and compartmentation in vacuoles is suggested to be of major importance in the carbon metabolism of guard cells (Tallman 1992). We suggest that a high degree of metabolic similarity may exist between guard cells and these trichomes particularly since both are epidermal specializations and at maturity are symplastically isolated from adjacent mesophyll and epidermal cells. Further, the basal cells have small chloroplasts (Lazzaro and Thomson 1989) and we suggest that the synthesis of malate and its compartmentation in the vacuolar complex probably occurs predominantly in the basal cells. In addition, since uptake and internalization of Lucifer yellow occurs at the lower stalk walls, the diffusion pathway of solute within the continuum is directionally limited and vectorially upward.

Acknowledgement

This research was supported by a grant from the Swedish Natural Sciences Research Council to Mark Lazzaro.

References

Cai G, Bartalesi A, Delcasino C, Moscatelli A, Tiezzi A, Cresti M (1993) The kinesin-immunoreactive homologue from *Nicotiana tabacum* pollen tubes – biochemical properties and subcellular localization. Planta 191: 496–506

Cole L, Coleman J, Kearns M, Morgan G, Hawes C (1991) The organic anion transport inhibitor, probenecid, inhibits the transport of Lucifer yellow at the plasma membrane and the tonoplast in suspension cultured plant cells. J Cell Sci 99: 545–555

Ding B, Turgeon R, Parthasarathy MV (1992) Substructure of freeze-substituted plasmodesmata. Protoplasma 169: 28–41

Dong Z, McCully ME, Canny MJ (1994) Retention of vacuole contents of plant cells during fixation. J Microsc 175: 222–228

Gunning BES, Overall RL (1983) Plasmodesmata and cell-to-cell transport in plants. Bioscience 33: 260–265

Knebel W, Quader H, Schnepf E (1990) Mobile and immobile endoplasmic reticulum in onion bulb epidermis cells: short- and long-term observations with a confocal laser scanning microscope. Eur J Cell Biol 52: 328–340

Kohno T, Shimmen T (1988) Accelerated sliding of pollen tube organelles along Characeae actin bundles regulated by calcium. J Cell Biol 106: 1539–1543

Lauter DJ, Munns DN (1986) Water loss via the glandular trichomes of chickpea (*Cicer arietinum*). J Exp Bot 37: 640–649

Lazzaro MD, Thomson WW (1989) Ultrastructure of organic acid secreting trichomes of chickpea (*Cicer arietinum*). Can J Bot 67: 2669–2677

– – (1992a) Endocytosis of lanthanum nitrate in the organic acid secreting trichomes of chickpea (*Cicer arietinum* L.). Am J Bot 79: 1113–1118

– – (1992b) Ultrastructural localization of calcium in the organic acid secreting trichomes of chickpea (*Cicer arietinum*). Can J Bot 70: 2319–2325

– – (1995) Seasonal variation in hydrochloric acid, malic acid, and calcium ions secreted by the trichomes of chickpea (*Cicer arietinum*). Physiol Plant 94: 291–297

Liebe S, Quader H (1994) Myosin in onion (*Allium cepa*) bulb scale epidermal cells: involvement in dynamics of organelles and endoplasmic reticulum. Physiol Plant 90: 114–124

Liu GQ, Cai G, Del Casino C, Tiezzi A, Cresti M (1994) Kinesin-related polypeptide is associated with vesicles from *Corylus avellana* pollen. Cell Motil Cytoskeleton 29: 155–166

Lucas WJ, Ding B, van der Schoot C (1993) Plasmodesmata and the supracellular nature of plants. New Phytol 125: 435–476

McCully ME, Canny MJ (1985) The stabilization of labile configurations of plant cytoplasm by freeze-substitution. J Microsc 139: 27–33

Martinoia E (1992) Transport processes in vacuoles of higher plants. Bot Acta 105: 232–245

Mersey B, McCully ME (1978) Monitoring of the course of fixation in plant cells. J Microsc 114: 49–76

Mitsui H, Yamaguchi-Shinozaki K, Shinozaki K, Nishikawa K, Takahashi H (1993) Identification of a gene family (*kat*) encoding kinesin-like proteins in *Arabidopsis thaliana* and characterization of secondary structure of KatA. Mol Gen Genet 238: 362–368

O'Brien TP, Kuo J, McCully ME, Zee SY (1973) Coagulant and non-coagulant fixation of plant cells. Aust J Biol Sci 26: 1231–1250

Oparka KJ (1991) Uptake and compartmentation of fluorescent probes by plant cells. J Exp Bot 42: 565–579

– Prior DAM (1988) Movement of Lucifer yellow CH in potato tuber storage tissues: a comparison of symplastic and apoplastic transport. Planta 176: 533–540

– Murant EA, Wright KM, Prior DAM, Harris N (1991) The drug probenecid inhibits the vacuolar accumulation of fluorescent anions in epidermal cells. J Cell Sci 99: 557–563

– Wright KM, Murant EA, Allan EJ (1993) Fluid-phase endocytosis – do plants need it. J Exp Bot 44: 247–255

Overall RL, Wolfe J, Gunning BES (1982) Intercellular communication in *Azolla* roots: I. Ultrastructure of plasmodesmata. Protoplasma 111: 134–150

Quader H, Hofmann A, Schnepf E (1989) Reorganization of the endoplasmic reticulum in epidermal cells of onion bulb scales after cold stress: involvement of cytoskeletal elements. Planta 177: 273–280

Robards AW, Lucas WJ (1990) Plasmodesmata. Annu Rev Plant Physiol Plant Mol Biol 41: 369–419

Robinson DG, Hedrich R (1990) Endocytosis in plants. Physiol Plant 79: 96–104

– – (1991) Vacuolar Lucifer yellow uptake in plants: endocytosis or anion transport; a critical opinion. Bot Acta 104: 257–264

Salitz A, Schmitz K (1989) Influence of microtubule and microfilament inhibitors applied by immersion and microinjection on circulation streaming in the staminal hairs of *Tradescantia blossfeldiana*. Protoplasma 153: 37–45

Schnepf E (1965) Licht- und elektronmikroskopische Beobachtungen an den Trichom-Hydathoden von *Cicer arietinum*. Z Pflanzenphysiol 53: 245–254

Shepherd VA, Orlovich DA, Ashford AE (1993a) A dynamic continuum of pleiomorphic tubules and vacuoles in growing hyphae of a fungus. J Cell Sci 104: 495–507

– – – (1993b) Cell to cell transport via motile tubules in growing hyphae of a fungus. J Cell Sci 105: 1173–1178

Srivastava CP, Srivastava RP (1989) Screening for resistance to gram pod borer, *Heliothis armigera* (Hubner), in chickpea (*Cicer arietinum* L.) genotypes and observations on its mechanism of resistance in India. Insect Sci Appl 10: 253–258

Stewart WW (1978) Functional connections between cells as revealed by dye coupling with a highly fluorescent naphthalimide tracer. Cell 14: 741–759

Tallman G (1992) The chemiosmotic model of stomatal opening revisited. Crit Rev Plant Sci 11: 35–57

Tiezzi A, Moscatelli A, Cai G, Bartalesi A, Cresti M (1992) An immunoreactive homologue of mammalian kinesin in *Nicotiana tabacum* pollen tubes. Cell Motil Cytoskeleton 21: 132–137

Thomson WW, Platt KA, Campbell NA (1973) The use of lanthanum to delineate the apoplastic continuum in plants. Cytobios 8: 57–62

White RG, Badelt K, Overall RL, Vesk M (1994) Actin associated with plasmodesmata. Protoplasma 180: 169–184

Wright KM, Davies TGE, Steele SH, Leigh RA, Oparka KJ (1992) Development of a probenecid-sensitive Lucifer yellow transport system in vacuolating oat aleurone protoplasts. J Cell Sci 102: 133–139

Vale RD, Schnapp BJ, Mitchison T, Steuer E, Reese TP, Sheetz MP (1985) Different axoplasmic proteins generate movement in opposite directions along microtubules in vitro. Cell 43: 623–632