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THE PREPROPHASE BAND: POSSIBLE INVOLVEMENT IN THE FORMATION OF THE CELL WALL

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SUMMARY

Numerous vesicles were observed among the microtubules of the 'preprophase' band in prophase cells from root tips of *Allium cepa*. The content of these vesicles looks similar to the matrix of adjacent cell walls, and these vesicles often appear to be involved in exocytosis. In addition, the cell walls perpendicular to the plane of (beneath) the preprophase band are often differentially thickened compared to the walls lying parallel to the plane of the band. Our interpretation of these observations is that the preprophase band may direct or channel vesicles containing precursors of the cell wall to localized regions of wall synthesis. The incorporation of constituents of the cell wall into a narrow region defined by the position of the preprophase band may be a mechanism that ensures unidirectional growth of meristematic cells.

INTRODUCTION

A preprophase band of microtubules (MTs) has been observed in a number of higher plants prior to the onset of somatic cell division (Pickett-Heaps & Northcote, 1966*a*, *b*; Burgess & Northcote, 1967, 1968, 1969; Esau & Gill, 1969; Pickett-Heaps, 1969*a–c*; Evert & Deshpande, 1970; Palevitz & Hepler, 1974). This band of MTs is located in the cortical cytoplasm in the plane of the future cell plate.

Although the preprophase band may accurately predict the plane of cell division (Pickett-Heaps & Northcote, 1966*a*, *b*), the function of this cortical band of MTs is not clear. Pickett-Heaps (1974) considers the band to be '. . . one response of the premitotic cell to the factors inducing polarization' and suggests that the subunits of the MTs are reused in the formation of the spindle. Palevitz & Hepler (1974) and Hepler & Palevitz (1974) propose that the MTs of the preprophase band '. . . may result from and thus indicate a morphogenetically important cortical initiation site' (i.e. a microtubule-organizing centre) which ultimately controls the final position of such cellular components as the spindle and phragmoplast. They do not, however, feel that the MTs of the band are necessarily an important component of this putative initiating site.

In contrast to these passive or non-functional roles for the preprophase band, we present evidence suggesting that the preprophase band functions in localized deposition of cell wall material. The incorporation of material into the cell wall in a narrow

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region defined by the position of the preprophase band may ensure unidirectional growth of meristematic cells (see Discussion).

MATERIALS AND METHODS

Root tips of *Allium cepa* were fixed for 1–2 h in 3 % glutaraldehyde in 0·05 M phosphate buffer (pH 6·8) at room temperature or at 4 °C. Following a buffer wash, the root tips were postfixed with 2 % osmium tetroxide in the same buffer. The material was then dehydrated in a graded acetone series or in a graded ethanol series to acetone and embedded in Epon-Araldite. Silver-gold sections were cut with a diamond knife on an LKB 4801 A ultramicrotome. Sections were mounted on Formvar-coated grids and stained with uranyl acetate and lead citrate. Observations were made with an AEI EM6B electron microscope at 60 kV.

We have found that fixation in the cold is superior to fixation at room temperature because the latter invariably results in a finely granular precipitate through the cytoplasm.

OBSERVATIONS

Although we were unable to identify a separate preprophase stage in cells from root tips of *A. cepa*, a cortical band of MTs, which we shall refer to as the 'preprophase band' in deference to earlier terminology (Pickett-Heaps & Northcote, 1966*a*), was observed in cells that were obviously in prophase (Figs. 1, 3). For this reason, we are inclined to agree with Burgess (1969, 1970) that this cortical band of MTs may be a characteristic feature of prophase rather than preprophase.

The preprophase band seen in our preparations consists of several layers of MTs located in close proximity to the plasma membrane (Figs. 2, 5, 7–11). These MTs lie in a plane that is assumed to correspond to the future plane of cell division (Pickett-Heaps & Northcote, 1966*a*, *b*) and are oriented roughly parallel to the plasma membrane. In tangential sections, the MTs of the preprophase band often appear to terminate in or on the plasma membrane (Figs. 8, 9, 11), suggesting that the MTs may be oriented at a slight angle to the surface of the cell, rather than strictly parallel to it. MTs of the band usually appear in cross-section when a root tip is sectioned longitudinally (Figs. 2, 7) and in longitudinal section when a root tip is sectioned transversely (Fig. 5) (Pickett-Heaps & Northcote, 1966*a*). Since most of the cells of a root

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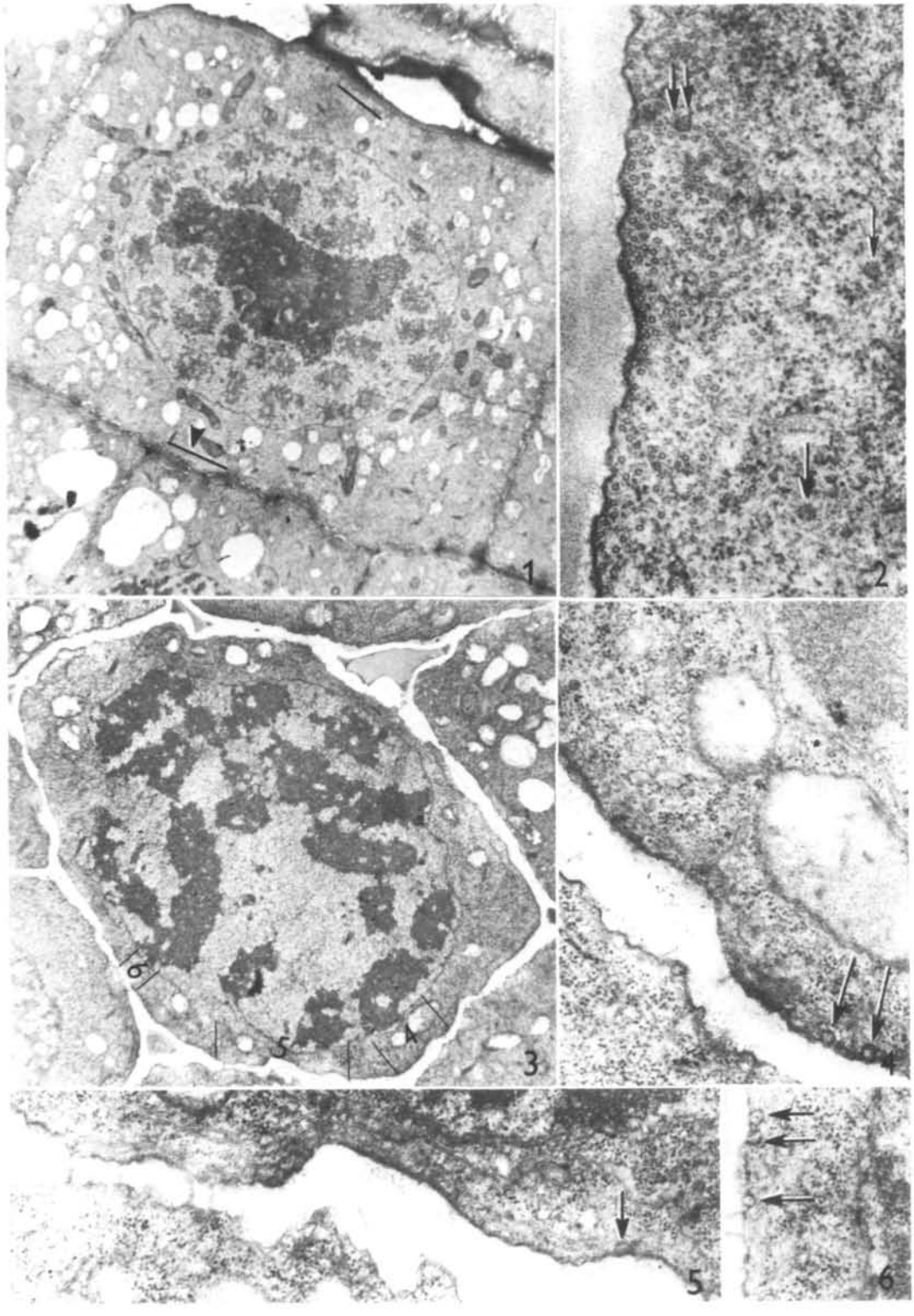
Fig. 1. Prophase cell from a root tip sectioned longitudinally. MTs of the preprophase band are found in a localized area of cortical cytoplasm under the radial walls (areas marked with bars). Note the relative thickness of the radial walls compared to the end walls. The region denoted by an arrowhead is shown at higher magnification in Fig. 2. × 7000.

Fig. 2. A portion of the preprophase band from the cell in Fig. 1. Several vesicles can be seen in this micrograph. One is found among the MTs of the band (paired arrows) and others can be seen in close proximity to the band (single arrows). × 40 000.

Fig. 3. Prophase cell from a root tip sectioned transversely. Note the typically thick radial walls. The areas labelled *4*, *5* and *6* between lines are shown at higher magnification in Figs. 4–6 respectively. × 7000.

Figs. 4–6. MTs of the preprophase band are seen in longitudinal view in the cortical cytoplasm and are oriented approximately parallel to the plasma membrane. Arrows indicate sites at which vesicles seem to be fusing with the plasma membrane. × 40 000.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tip divide transversely to the long axis of the root, and assuming that the MTs of the band lie in the plane of the future cell plate, one would predict this general orientation for MTs of the preprophase band.

Vesicles, similar to those seen among the MTs of the phragmoplast (Ledbetter & Porter, 1963; Hepler & Jackson, 1968; Bajer, 1968; Palevitz & Hepler, 1974) were consistently seen among the MTs of the preprophase band (Figs. 2, 7–10). In fact, it was often possible to locate the position of the preprophase band by first locating an accumulation of vesicles near the plasma membrane. In general, the electron density of the contents of these vesicles corresponds closely to the electron density of the cell walls (Figs. 7–9). Occasionally, we observed vesicles which seemed to be fusing with the plasma membrane under the preprophase band (Figs. 4–6, 10, 11). In addition, the walls perpendicular to the plane of the preprophase band, i.e. the walls beneath the preprophase band, are often differentially thickened compared to the walls lying parallel to the plane of the band (Fig. 1). This general thickening of the walls beneath the preprophase band might be expected if the band is a site of exocytosis of precursors of the cell wall.

DISCUSSION

Subsequent to the identification of MTs in the electron microscope, a number of investigators have presented evidence that MTs may be involved in the formation of cell walls (Hepler & Newcomb, 1964; Pickett-Heaps, 1966, 1967, 1968; Pickett-Heaps & Northcote, 1966*c*; Hepler & Fosket, 1971; Maitra & De, 1971; Nelmes, Preston & Ashworth, 1973; Hepler & Palevitz, 1974). It is generally thought that cortical MTs may direct vesicles containing precursors of the cell wall to sites at which synthesis of the wall is occurring (Pickett-Heaps, 1968; Maitra & De, 1971; Hepler & Palevitz, 1974). In addition, MTs of the phragmoplast are thought to be responsible for directing vesicles containing constituents of the cell wall to the developing cell plate (Hepler & Jackson, 1967, 1968; Bajer, 1968; Hepler & Palevitz, 1974).

Although there is no direct evidence that MTs can move vesicles in plant cells, MTs apparently are actively involved in the movement of pigment granules in certain kinds of melanophores (Murphy & Tilney, 1974), and firm linkages can occur between MTs and vesicles in *Paramecium caudatum* (Allen, 1975).

Our proposal is that the MTs of the preprophase band direct or channel vesicles containing precursors of the cell wall to the plasma membrane where exocytosis

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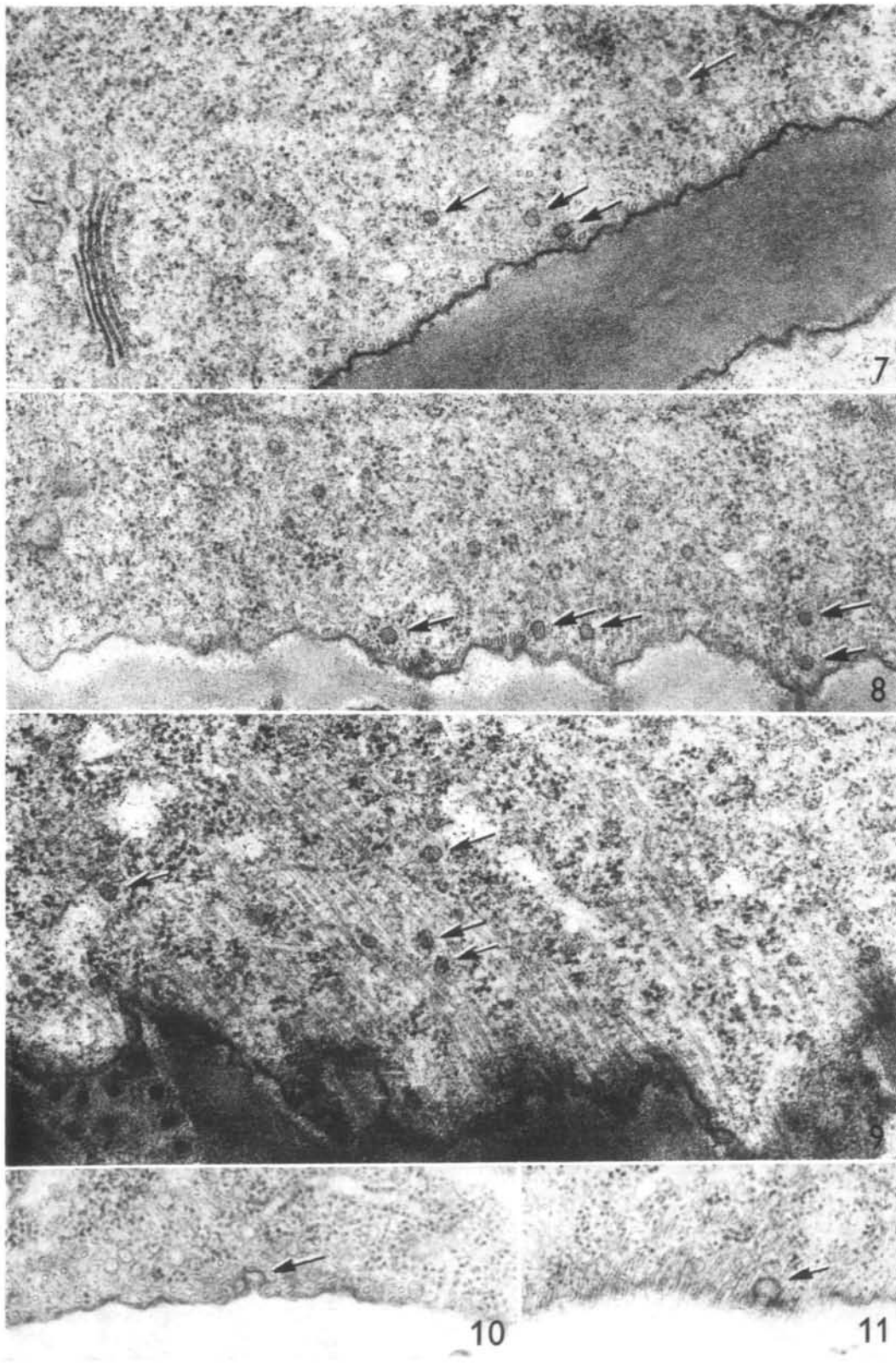
Figs. 7–11. Portions of the preprophase band from 5 different prophase cells in root tips that were sectioned longitudinally. In Figs. 7–9 arrows denote vesicles among the MTs of the band. The contents of the vesicles typically have a density similar to the cell wall as does the dictyosome in Fig. 7. Figs. 7 and 10 are near-sagittal sections of prophase cells and the MTs of the bands appear primarily in cross section. In Figs. 8, 9 and 11 the cells were cut tangentially, and the MTs are seen in more or less longitudinal section. When the band is sectioned in this fashion, the MTs appear to terminate in or on the plasma membrane. In Figs. 10 and 11 arrows point to vesicles that appear to be fusing with the plasma membrane. ×40000.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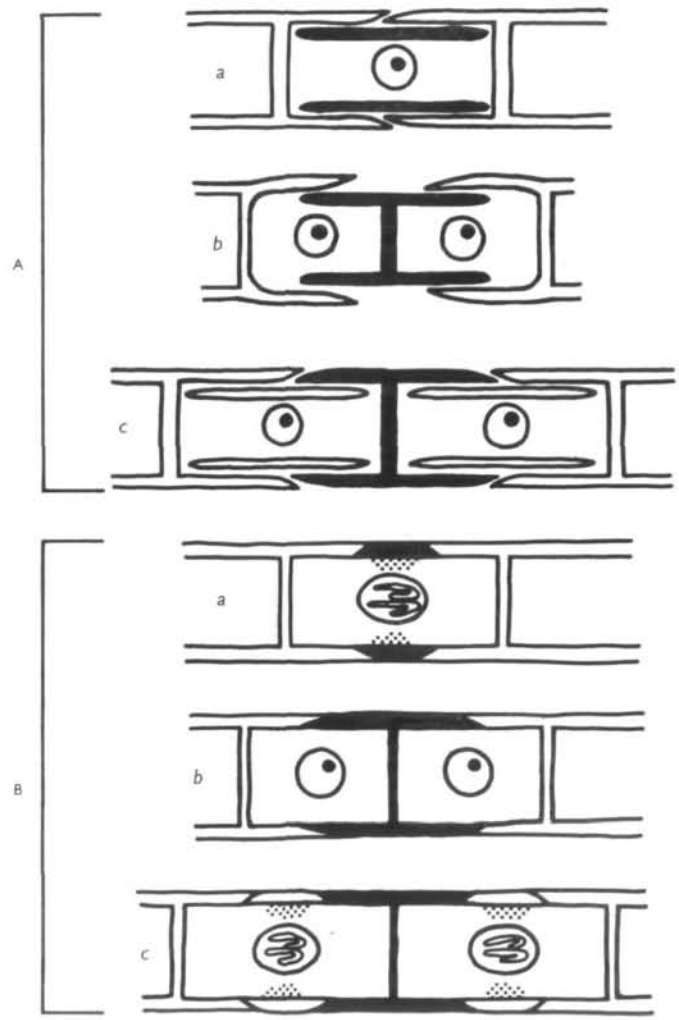

Fig. 12. Formation of H-shaped wall pieces in the green alga *Microspora* sp. (after Pickett-Heaps, 1973) compared with the hypothetical formation of H-shaped wall pieces in cells from root tips of *Allium cepa*. All views are longitudinal sections of columns of cells.

A. *Microspora*. *a*, deposition of a cylinder of wall material (shown in solid black) under the old radial walls during interphase. *b*, cytokinesis by the formation of a cell plate (shown in solid black) during telophase completes the H-shaped wall piece. *c*, as cells expand during interphase H-shaped wall pieces slip apart while new cylinders of wall material are laid down.

B. *Allium cepa*. *a*, deposition of a cylinder of wall precursors (shown in solid black) occurs in association with the 'preprophase' band of MTs (shown here as black dots) during late interphase or prophase of mitosis. *b*, formation of the cell plate (shown in solid black) during telophase completes the H-shaped wall piece. Cell elongation also begins. Assuming radial growth to be impossible because of the mechanical resistance of surrounding cells, only longitudinal growth along the slippage plane of the radial walls occurs. *c*, in succeeding prophases, new preprophase bands again direct or channel vesicles containing precursors of the cell wall to the plasma membrane, where exocytosis occurs, and a new cylinder of wall material is formed.

occurs and that the contents of the vesicles become inserted into the wall in a narrow band defined by the position of the preprophase band. If subsequent expansion of the cell walls of meristematic cells is more or less limited to this region of the wall and if expansion can occur only in the plane of the wall, the result would be unidirectional growth of the cell. Thus, the preprophase band could be one aspect of a general mechanism for controlling the direction of growth of higher plant cells.

Examination of micrographs published by other investigators provides additional evidence in support of our hypothesis. In many instances vesicles may be observed among the MTs of the preprophase band (Burgess & Northcote, 1969, fig. 4; Pickett-Heaps, 1969*a*, figs. 8–10, 11A; Burgess, 1970, figs. 6, 10–12; Palevitz & Hepler, 1974, fig. 7), and a similar thickening of the walls beneath the preprophase band is evident in a number of other micrographs (Pickett-Heaps & Northcote, 1966*a*, fig. 2; Pickett-Heaps, 1969*a*, figs. 12, 14, 18; Palevitz & Hepler, 1974, fig. 5).

The model that we propose for the formation of cell walls in higher plants is similar in many respects to the pattern of wall formation in the green alga *Microspora* sp. (Pickett-Heaps, 1973) (Fig. 12A). Briefly, in *Microspora* and related algae, the interphase cell is enclosed in 2 overlapping, 'H-shaped wall pieces' (Pickett-Heaps, 1973). During interphase, a new cylinder of wall material is laid down just inside the overlapping radial walls. At the time of cytokinesis, a new cross-wall is deposited in the mid-plane of this cylinder to complete the new H-shaped wall piece. As the daughter cells expand, the H-shaped wall pieces move apart.

In comparison, the cell walls in root tips of *A. cepa* are not obviously segmented but we suggest that a comparable cylinder of wall material is laid down during late interphase or prophase (Fig. 12B). Although this material may be inserted into the cell wall at prophase, the actual expansion of the wall could take place gradually at later stages, including the succeeding interphase. The formation of the cell plate at telophase would complete the new H-shaped wall piece.

With respect to the evolution of higher plants, Pickett-Heaps (1975) recently suggested that higher plants may have evolved from algal ancestors with a pattern of wall formation like that exhibited by *Microspora* sp. However, he pointed out that there was no evidence that a similar pattern of wall formation occurs in higher plants. If our interpretation of the function of the preprophase band is correct, then the pattern of wall formation in higher plants may be similar to that found in *Microspora*.

We consider our proposal for the function of the preprophase band of MTs to be attractive for several reasons. First, the suggestion that the MTs of the preprophase band serve no function and merely reflect the high concentration of tubulin occurring at this time (Hepler & Palevitz, 1974; Palevitz & Hepler, 1974; Pickett-Heaps, 1974) seems unsatisfactory. In a teleological sense it appears unnecessarily 'wasteful' for a cell to expend energy reserves building MTs that are to serve no function. Although it is by no means true that all structures have a function (or that we can correctly perceive that function, perhaps), it does seem unlikely that a structure as complex and ordered and as widely distributed among higher plants as the preprophase band would have no function. Secondly, although most of the observations concerning the preprophase band and the speculation concerning its function have been made on

differentiating cells that divide asymmetrically, our model could apply to the function of the preprophase band in all cells in which one is found, and not just to those cells which undergo asymmetric divisions. And, thirdly, our proposal is compatible with the observations of other investigators (Hepler & Palevitz, 1974; Palevitz & Hepler, 1974; Pickett-Heaps, 1974).

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REFERENCES

ALLEN, R. D. (1975). Evidence for firm linkages between microtubules and membrane-bounded vesicles. *J. Cell Biol.* **64**, 497–503.

BAJER, A. (1968). Fine structure studies on phragmoplast and cell plate formation. *Chromosoma* **24**, 383–417.

BURGESS, J. (1969). Two cytoplasmic features of prophase in wheat root cells. *Planta* **87**, 259–270.

BURGESS, J. (1970). Interactions between microtubules and the nuclear envelope during mitosis in a fern. *Protoplasma* **71**, 77–89.

BURGESS, J. & NORTHCOTE, D. H. (1967). A function of the preprophase band of microtubules in *Phleum pratense*. *Planta* **75**, 319–326.

BURGESS, J. & NORTHCOTE, D. H. (1968). The relationship between the endoplasmic reticulum and microtubular aggregation and disaggregation. *Planta* **80**, 1–14.

BURGESS, J. & NORTHCOTE, D. H. (1969). Action of colchicine and heavy water on the polymerization of microtubules in wheat root meristem. *J. Cell Sci.* **5**, 433–451.

ESAU, K. & GILL, R. H. (1969). Structural relations between nucleus and cytoplasm during mitosis in *Nicotiana tabacum* mesophyll. *Can. J. Bot.* **47**, 581–591.

EVERT, R. F. & DESHPANDE, B. P. (1970). An ultrastructural study of cell division in the cambium. *Am. J. Bot.* **57**, 942–961.

HEPLER, P. K. & FOSKET, D. E. (1971). The role of microtubules in vessel member differentiation in *Coleus*. *Protoplasma* **72**, 213–236.

HEPLER, P. K. & JACKSON, W. T. (1967). Microtubules, mitochondria, and endoplasmic reticulum associated with cell plate formation in the endosperm of *Haemanthus katherinae* Baker. *J. Cell Biol.* **35**, 56A (Abstr.).

HEPLER, P. K. & JACKSON, W. T. (1968). Microtubules and early stages of cell-plate formation in the endosperm of *Haemanthus katherinae* Baker. *J. Cell Biol.* **38**, 437–446.

HEPLER, P. K. & NEWCOMB, E. H. (1964). Microtubules and fibrils in the cytoplasm of *Coleus* cells undergoing secondary wall deposition. *J. Cell Biol.* **20**, 529–533.

HEPLER, P. K. & PALEVITZ, B. A. (1974). Microtubules and microfilaments. *A. Rev. Pl. Physiol.* **25**, 309–362.

LEDBETTER, M. C. & PORTER, K. R. (1963). A 'microtubule' in plant cell fine structure. *J. Cell Biol.* **19**, 239–250.

MAITRA, S. C. & DE, D. N. (1971). The role of microtubules in secondary thickening of differentiating xylem element. *J. Ultrastruct. Res.* **34**, 15–22.

MURPHY, D. B. & TILNEY, L. G. (1974). The role of microtubules in the movement of pigment granules in teleost melanophores. *J. Cell Biol.* **61**, 757–779.

NELMES, B. J., PRESTON, R. D. & ASHWORTH, D. (1973). A possible function of microtubules suggested by their abnormal distribution in rubbery wood. *J. Cell Sci.* **13**, 741–751.

PALEVITZ, B. A. & HEPLER, P. K. (1974). The control of the plane of division during stomatal differentiation in *Allium*. I. Spindle reorientation. *Chromosoma* **46**, 297–326.

PICKETT-HEAPS, J. D. (1966). Incorporation of radioactivity into wheat xylem walls. *Planta* **71**, 1–14.

PICKETT-HEAPS, J. D. (1967). The effects of colchicine on the ultrastructure of dividing plant cells, xylem wall differentiation and distribution of cytoplasmic microtubules. *Devl Biol.* **15**, 206–236.

PICKETT-HEAPS, J. D. (1968). Xylem wall deposition. Radioautographic investigations using lignin precursors. *Protoplasma* **65**, 181–205.

PICKETT-HEAPS, J. D. (1969*a*). Preprophase microtubule bands in some abnormal mitotic cells of wheat. *J. Cell Sci.* **4**, 397–420.

PICKETT-HEAPS, J. D. (1969*b*). Preprophase microtubules and stomatal differentiation; some effects of centrifugation on symmetrical and asymmetrical cell division. *J. Ultrastruct. Res.* **27**, 24–44.

PICKETT-HEAPS, J. D. (1969*c*). Preprophase microtubules and stomatal differentiation in *Commelina cyanea*. *Aust. J. biol. Sci.* **22**, 375–391.

PICKETT-HEAPS, J. D. (1973). Cell division and wall structure in *Microspora*. *New Phytol.* **72**, 347–355.

PICKETT-HEAPS, J. D. (1974). Plant microtubules. In *Dynamic Aspects of Plant Ultrastructure* (ed. A. W. Robards), pp. 219–255. London: McGraw-Hill.

PICKETT-HEAPS, J. D. (1975). *Evolution in Green Algae*. Seminar presented to Department of Botany and Plant Pathology, Colorado State University, Fort Collins, CO., Oct. 29.

PICKETT-HEAPS, J. D. & NORTHCOTE, D. H. (1966*a*). Organization of microtubules and endoplasmic reticulum during mitosis and cytokinesis in wheat meristems. *J. Cell Sci.* **1**, 109–120.

PICKETT-HEAPS, J. D. & NORTHCOTE, D. H. (1966*b*). Cell division in the formation of the stomatal complex of the young leaves of wheat. *J. Cell Sci.* **1**, 121–128.

PICKETT-HEAPS, J. D. & NORTHCOTE, D. H. (1966*c*). Relationship of cellular organelles to the formation and development of the plant cell wall. *J. exp. Bot.* **17**, 20–26.

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