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Edited by
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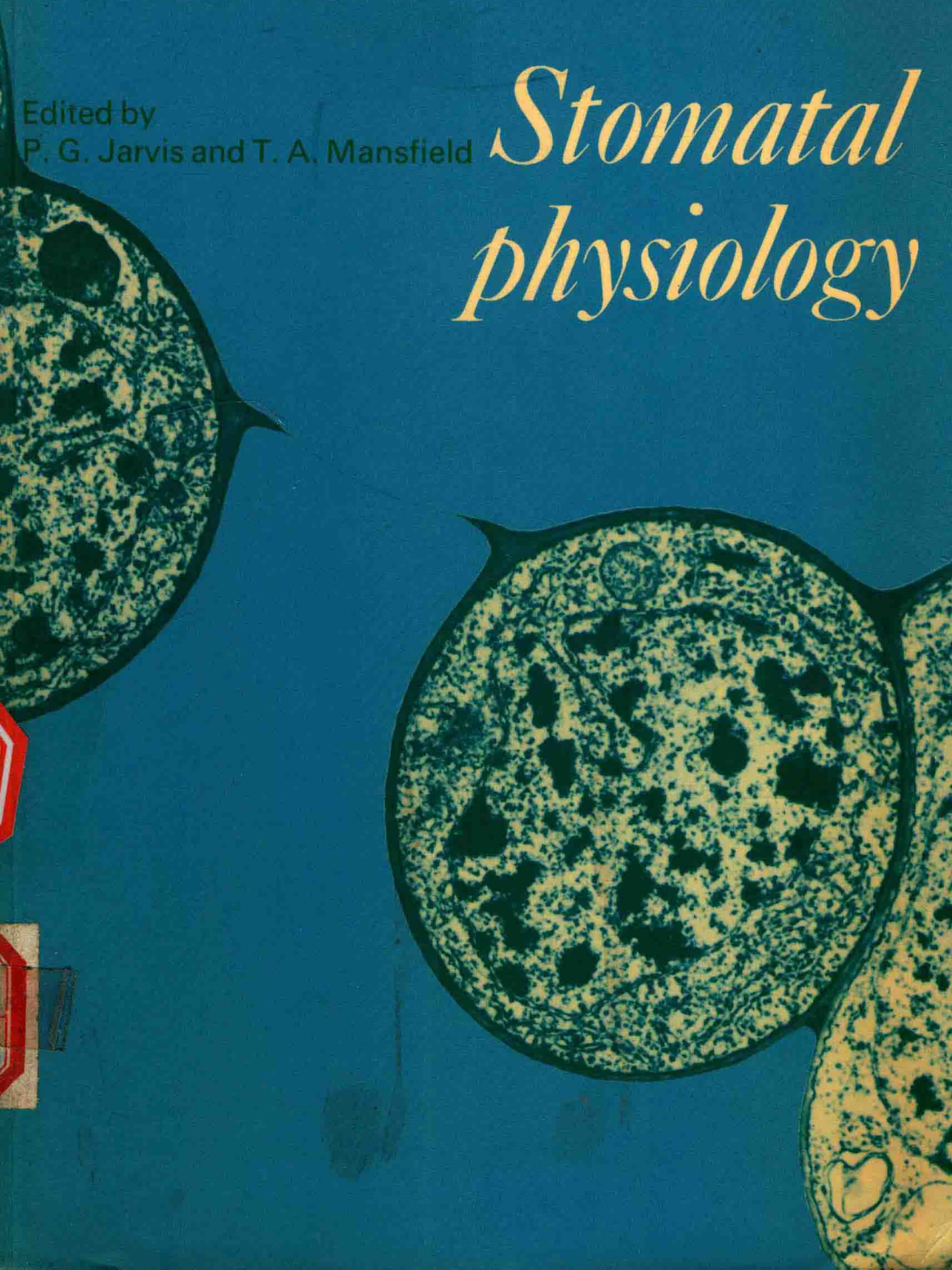
Stomatal physiology



STOMATAL PHYSIOLOGY

Edited by

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PREFACE

The contributors to this volume were the invited speakers in a seminar organized by the Society for Experimental Biology at the University of Lancaster on 19–20 December 1979. The object of the seminar was to present the many facets of a subject that has assumed increasing importance over the past two decades. Stomatal physiology is now covered in some depth in many undergraduate courses, and is encountered by many graduate students and researchers whose main interests are in other areas of plant biology. It is hoped that this collection of papers will be helpful to many people in these categories. Our contributors have not only presented results of their own recent research, but have introduced their topics for the non-specialist.

CONTENTS

B. A. PALEVITZ

The structure and development of stomatal cells

Introduction

Botanists have long been intrigued by the relationship between structure and function in plant cells, including those that comprise the stomatal apparatus. Because the architecture and content of guard cells is so obviously important in their behaviour, it is pertinent to inquire into their structure and the mechanisms that govern their differentiation. Furthermore, a thorough understanding of the structure and development of guard cells can only be obtained with comprehensive information at the cell biological level. Such information can assist us in interpreting how developing cells respond to external signals emanating from the environment and from other cells, and what internal processes control differentiation.

Guard cells are useful as model systems for the study of basic problems in cell biology and development. The processes of growth and cell shaping, and the 'cooperative' interaction between adjacent cells during development and function, have long intrigued biologists. These can be successfully studied using stomatal cells. Guard cells in particular offer favourable material to attack problems such as the control of osmotic and ionic fluxes at membranes, the control of cell surface activity during the deposition of extracellular macromolecules (cell wall), and the role of motile or cytoskeletal elements (microtubules and microfilaments) in the regulation of plasma membrane activity and wall formation.

The subject of stomatal structure has been reviewed recently (Allaway & Milthorpe, 1976). In this article, I shall review various aspects of stomatal anatomy, with an emphasis on wall formation in developing guard cells. The reader should gain an appreciation for the timing and relationships of various formative processes to each other during maturation. Hopefully, the reader will also sense the value of stomata in obtaining information of more general significance at the cellular level.

Cell division

The formation of stomatal cells involves the precise placement of division planes in parent cells. The mechanisms that determine the orientation of the new cell plate in plants have always been of intense interest, and much attention has been directed at the stomatal apparatus as a model system. The formation of guard mother cells and subsidiary cells usually involves asymmetric division in which the nucleus migrates to a specific site in the parent cell before dividing (reviewed by Hepler & Palevitz, 1974). In addition, the orientation of the new cell plate is often radically different from that resulting from the division of other epidermal cells. Little is known about the mechanisms underlying these phenomena. Considerable circumstantial evidence indicates that cells such as guard mother cells somehow control mitotic activity and the placement of division planes in neighbouring cells (e.g. subsidiary cells) by some field effect involving chemical gradients. Undoubtedly motility-producing macromolecules (microtubules, actin) are involved in the requisite movements, but which ones operate in each case is unclear. It is known that certain inhibitors such as colchicine and cytochalasin B may interfere with nuclear migrations and polarity phenomena (Hepler & Palevitz, 1974). In the division of guard mother cells of *Allium cepa* L., the spindle apparatus is oriented obliquely through anaphase, leading to the separation of daughter chromosomes into opposite corners of the cell (Palevitz & Hepler, 1974; Palevitz, 1980). A specific, directed movement of the daughter nuclei and forming cell plate then ensues, leading to the proper longitudinal placement of the plate. It appears that both microtubules and microfilaments are involved, because the process is sensitive to agents which affect both structures (colchicine, cytochalasin B, phalloidin). This same process also occurs in the guard mother cells of other *Allium* species as well as in those of the Gramineae. A preprophase band of microtubules is known to mark the future division plane in these cells, but a role in actually orienting the cell plate does not seem likely because the band disappears long before telophase. Instead, the band may reflect a circumferential, morphogenetically-determined region of the plasma membrane that somehow interacts with the nucleus, spindle apparatus or forming cell plate during division (Hepler & Palevitz, 1974; Palevitz & Hepler, 1974). French & Paolillo (1975) reported that the plane of division in guard mother cells of moss sporophytes is influenced by physical contact with the calyptra. The significance of this observation in relation to the above movements is unclear. It is hoped that a solution to these long-perplexing processes will soon be forthcoming.

Cell shape and wall deposition in guard cells

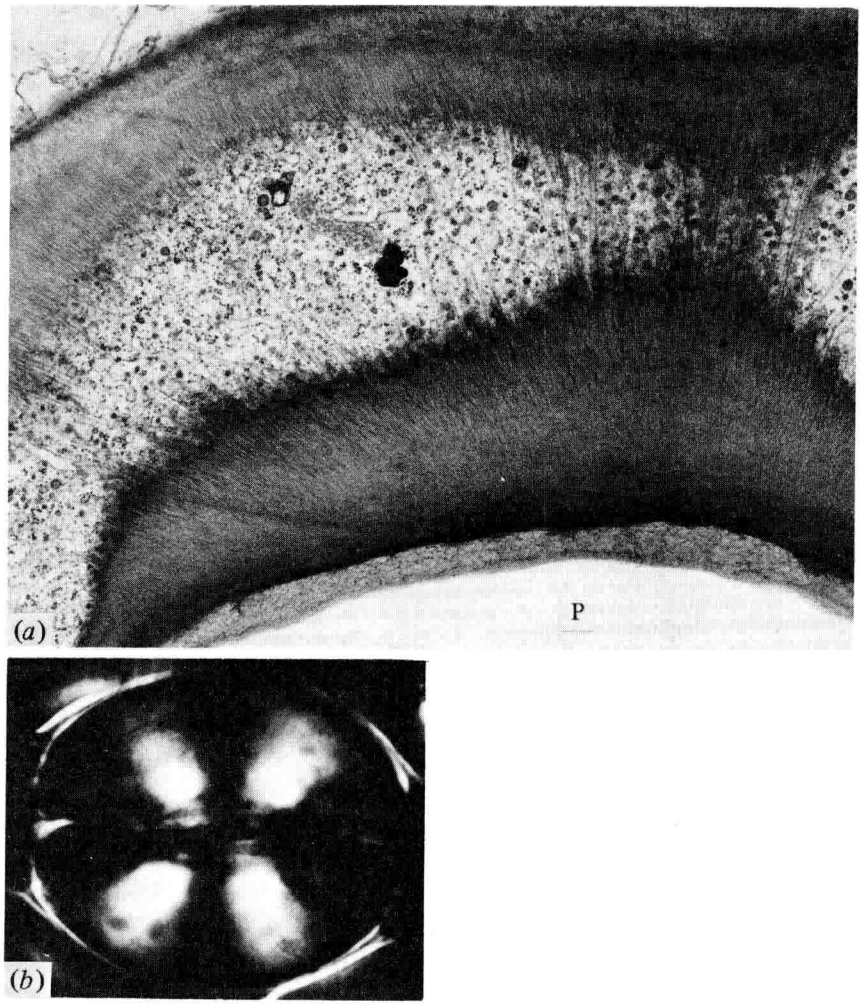
Perhaps the two most distinctive features of guard cells are their characteristic shapes and non-uniform wall thickenings. In plants, cell shape is governed by the surrounding cell wall. The mechanical properties of the wall in turn are in large part determined by the orientation of newly deposited cellulose microfibrils. In addition, a characteristic of many highly differentiated plant cells such as guard cells and tracheal elements is the deposition of thick, often distinctly patterned secondary walls in which the cellulose is also highly oriented. Evidence indicates that the pattern of wall deposition and the orientation of wall microfibrils in plant cells may be determined by the pattern and co-orientation of cortical microtubules adjacent to the plasma membrane (Hepler & Palevitz, 1974). Microtubules may exert this control by interacting with specific components in the plasma membrane, either indirectly through the active generation of shear in the plane of the fluid membrane or directly by linkages between microtubules and membrane-bound components such as cellulose-synthesizing complexes.

The mechanism governing the alignment of wall cellulose, however, has remained controversial, and co-orientation of microtubules and cellulose has not always been seen. One of the main reasons for lack of resolution of the problem in plants has been the choice of cells studied. What is clearly needed is a cell system which exhibits the combined characteristics of growth accompanied by shape change; the deposition of localized wall thickenings containing cellulose microfibrils, the orientation of which is precise and known; and ready accessibility enabling easy observation with light and electron microscopy. Guard cells fulfil all these criteria. In addition, a comprehensive literature exists on the physiology of these cells during gas exchange.

The patterns of wall thickening in guard cells vary amongst species. Typically the upper and lower paradermal walls in the vicinity of the pore are heavily thickened, but the amount of deposition on each can vary. The ventral wall bordering the pore is also thickened, though less so than the paradermal walls. The ventral and anticlinal end walls in some guard cells may also be thickened near their juncture. The common wall of the guard and subsidiary cells is relatively unthickened, as are other walls of subsidiary cells. In the elliptical or kidney-shaped guard cells of plants such as *Allium* and *Pisum*, the paradermal walls are thickened in a fan-like pattern that radiates away from the pore (Singh & Srivastava, 1973; Palevitz & Hepler, 1976). The ventral wall bordering the pore is also thickened. Wall microfibrils in these thickenings are oriented in a similar, radial manner; that is, they too radiate away from the pore site in a fan-like pattern (Fig. 1*a, b*).

Immediately following division of the precursor guard mother cell, the immature guard cells begin a marked change in shape. The first sign of differentiation in these cells is the assembly of microtubules at a site close to the plasma membrane and adjacent to the future pore in the common wall between sister guard cells (Palevitz & Hepler, 1976). Soon after the microtubules appear, the common wall thickens at this site. The new cellulose

Fig. 1. (*a*) Tangential section through a guard cell of *Allium cepa*. Note the thickened walls, the cuticle, and the radially-arranged microfibrils and microtubules (P, pore). Micrograph by M. Doohan & B. Palevitz. ×14300. (*b*). Polarization micrograph of similar *Allium* guard cells showing birefringence of aligned microfibrils. ×1000.



microfibrils are deposited in the radial, fan-like array described above. This alignment exactly parallels that of the population of cortical microtubules which appeared prior to wall thickening (Fig. 1*a*). Moreover, this orientation remains constant during differentiation, a point to be remembered later when we discuss the guard cells of grasses. Thus, it is proposed that microtubules determine both the localization of thickening and the orientation of cellulose. Soon after the initiation of the new wall layer, the cell begins to assume its characteristic kidney shape. The change in shape is produced by the continued growth of the cells constrained by the new, radial array of wall microfibrils. Colchicine, which leads to the breakdown of cortical microtubules, disrupts cell shape, the localization of wall thickening, and the orientation of the wall microfibrils. Thus, one of the early, determinative events in the differentiation of the guard cells of *Allium* is the precise, oriented assembly of microtubules at specific sites in the cell.

The guard cells of grasses such as timothy-grass (*Phleum pratense* L.) and maize are quite different from those of *Allium* (Kaufman, Petering, Yocum & Baic, 1970; Srivastava & Singh, 1972; Ziegler, Schmueli & Lange, 1974; Palevitz & Alones, 1977). At maturity, these cells are highly elongate and bone- or dumb-bell-shaped (Fig. 2*a, b*). The wall around the constricted midzone of each cell is heavily thickened (Fig. 2*a, c*). Early work, especially that by Ziegenspeck (1944), demonstrated that wall microfibrils in this region are axially oriented compared to the radial arrangement found in *Allium*. Because the shape and wall architecture in the guard cells of grasses is so different from that of elliptical cells, it would be instructive to compare differentiation in these species. Furthermore, in the grasses, the differentiation of guard cells is accompanied by the formation of adjacent subsidiary cells, and the two cell types function together in mature stomatal activity. It is of interest to know what effect if any such subsidiary cells have on neighbouring, growing guard cells and to what extent these cells interact during development.

Young guard cells of grasses, soon after division of the guard mother cells, have a characteristic shape – that is, their lateral walls curve inwards (Fig. 3*a*). This shape is even evident in the guard mother cells prior to division. Gradually, a pad-like thickening develops in the common wall between the guard cells in a manner similar to that of *Allium*. Electron microscopy at this early stage reveals ultrastructural detail also similar to that of *Allium*. The assembly of microtubules in a zone adjacent to the plasma membrane precedes the formation of this thickening (Fig. 5*a*). Both microtubules and wall cellulose radiate away from the site in a fan-like array as in *Allium*.

Guard cells continue to increase in size but retain their shape up to the

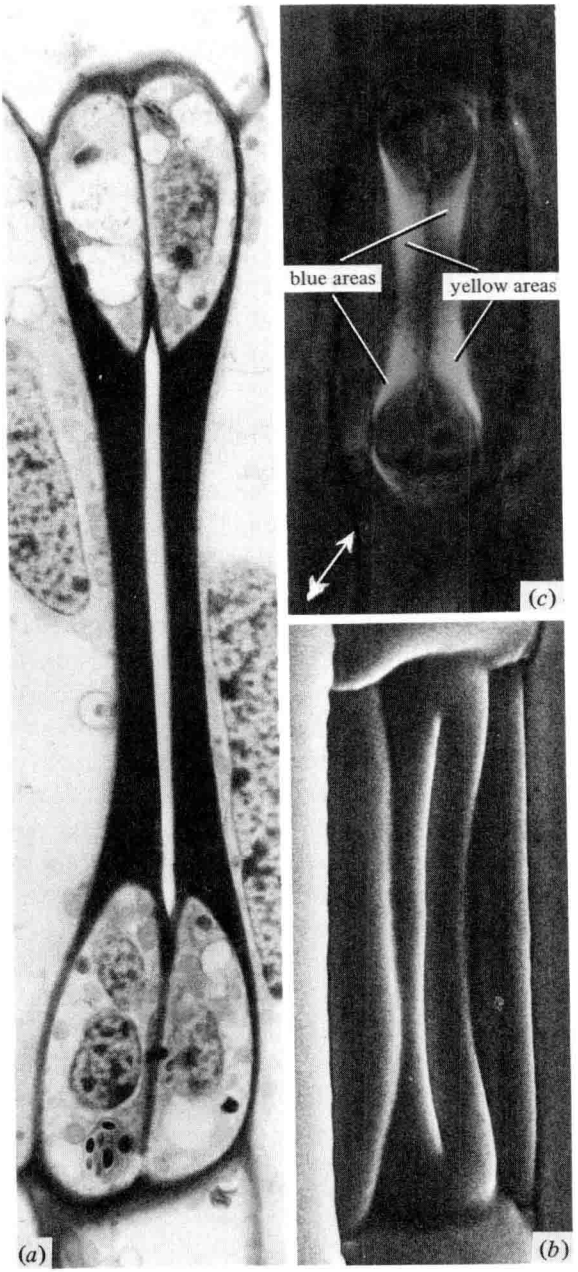


Fig. 2. (*a*). High voltage electron micrograph of a mature stomatal complex of timothy-grass. Note the slit-like pore and heavily thickened wall in the mid-zone of each bone-shaped guard cell. ×3500. (*b*). A scanning electron micrograph of a similar timothy-grass stomatal

time the pore is first formed. Then, a radical change in cell shape occurs at the time the pore first becomes evident with the light microscope. The lateral wall of the guard cells bulges out drastically as the cells assume an elliptical shape (Figs. 3*b*, 8). At the same time, the subsidiary cells flatten. Scanning electron microscopy as well as cross-sections of embedded cells show that the upper and lower paradermal walls bow out as well at this stage (Fig. 4). As the guard cells begin to bulge, there is a marked increase in the size of their vacuoles, indicating that active osmotic processes are responsible for the shape change (Figs. 3*b*, 8*b*). Electron microscopy shows that microtubules are still oriented radially, as in *Allium* (Fig. 5*b*). Indeed, the cells are now strikingly similar in overall appearance to the guard cells of *Allium*. Yet an important difference is also evident. At the time the pore opens, the new

Fig. 3. Representative sequence of differentiation in grass stomatal cells. (*a*) Very young cells of maize. ×1500. (*b*) Transient guard cell swelling stage in maize. ×1700. (*c*) Reconstruction of guard cells in timothy. ×1500. (*d*) Mature complex of timothy. ×1300.

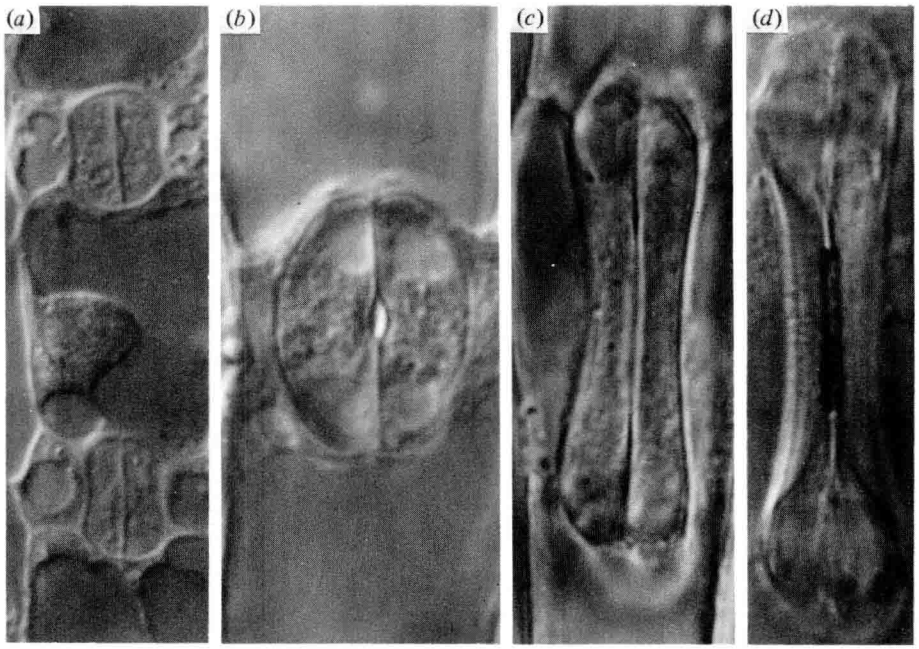


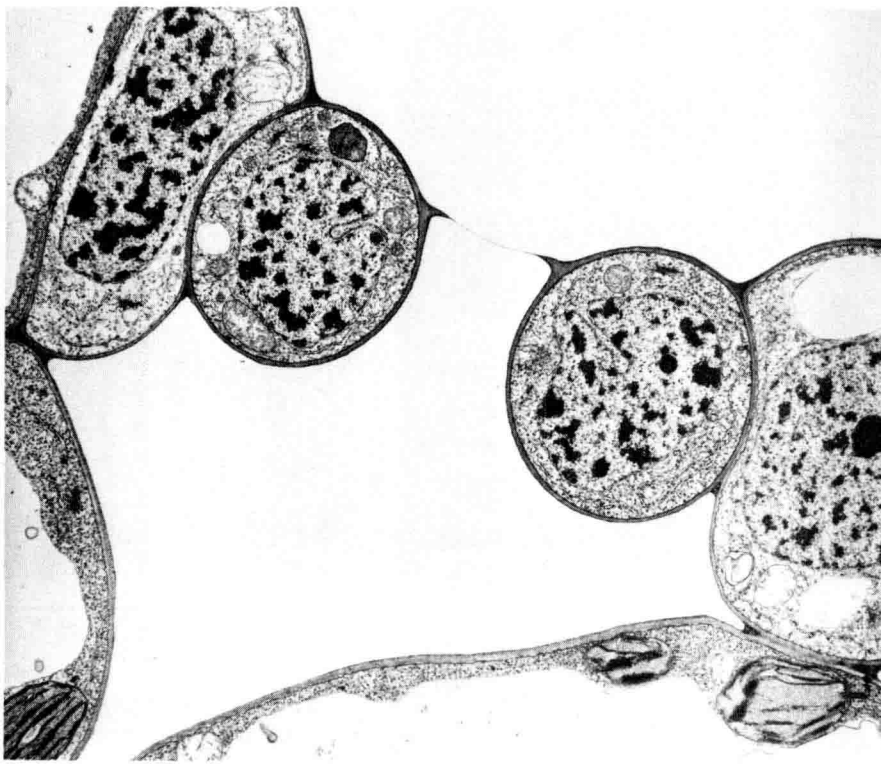
Fig. 2 continued
complex. Micrograph by M. Ledbetter & B. Palevitz. ×1200.
(*c*) Polarization micrograph of timothy-grass using a Red I Plate. By rotating the specimen relative to the plate major axis (bar), the resulting colours reveal wall microfibril orientation. ×1100.

wall is not nearly as thick as in *Allium* at the equivalent stage. Wall birefringence is also still hardly detectable, in contrast to *Allium*.

As stated above, grass guard cells are highly elongated and bone-shaped at maturity. But at this intermediate stage of differentiation, they assume a kidney shape. In fact, this kidney-like swelling is just a transient shape-change. The cells soon begin to reconstrict and elongate as the pore becomes long and slit-like (Fig. 3*c*).

What is happening to the cell wall at this time? We have already noted that the wall is not very thick in guard cells at the time of the transient shape-change. However, polarization optics shows a rapid, accelerated deposition of new wall material in the narrow mid-zone of each guard cell soon after reconstriction (Fig. 3*d*). This new wall layer flares out at the bulbous ends of the cell (Figs. 2*a*, 3*d*). The wall becomes very thick and birefringence retardation values eventually exceed those of *Allium*. Wall thickening is

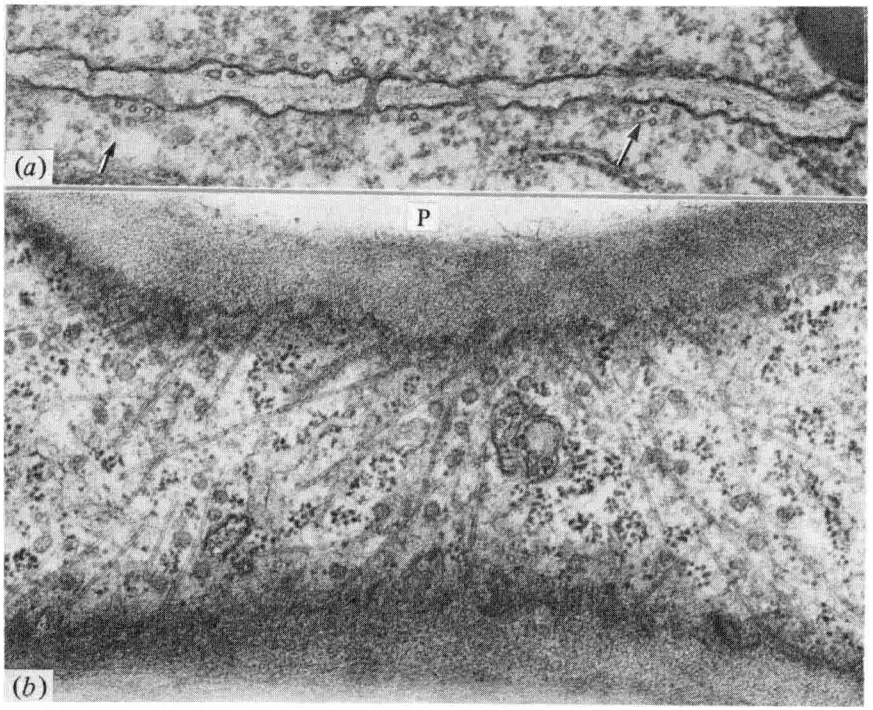
Fig. 4. Mid-cross-section through a timothy-grass complex at the transient guard cell swelling stage. ×5300.



accompanied by increased activity of the Golgi apparatus and the appearance of large numbers of vesicles in the cortical cytoplasm.

What about microtubule and microfibril orientation at this stage? As I have already mentioned, Ziegenspeck (1944) claimed that the cellulose in mature grass guard cells is axial in orientation; that is, it is aligned along the long axis of the cell and not radially as in *Allium* and other species with elliptical guard cells. Electron microscope and polarization analysis shows that indeed the cellulose deposited *late in differentiation* is *axial*. More precisely, the microfibrils are net axial – i.e., they are arrayed in a steep, criss-crossed pattern (Fig. 6*a*) which flares out at the bulbous ends of the cell. Most importantly, the cortical microtubules in such cells are also oriented in a steep, axial pattern (Fig. 6*a*). Cross-bridges can also be seen linking microtubules to the plasma membrane (Fig. 6*b*).

Fig. 5. (*a*). Mid-longitudinal section soon after cell division in timothy-grass. Note microtubules (arrows) in each guard cell near the plasma membrane bordering the common wall. ×39000.
(*b*). Mid-transient guard cell in tangential section. Note the mostly radial microtubules and wall microfibrils. A cuticle is not yet present. ×32000.



Our results above indicate that in grasses there is a shift or reorientation in microtubules, followed by cellulose, from radial to near axial, midway through differentiation when wall deposition rapidly accelerates. Indeed microtubules do seem to assume a more axial orientation just following the transient (i.e., in reconstricting cells). At first a few axial microtubules appear interspersed with the pre-existing radial array. Gradually more tubules appear in the new orientation as radial tubules decrease in number until the fully realigned arrangement is produced (Fig. 7). Thus, the shift in microtubule orientation precedes and/or accompanies the shift in cellulose orientation in the new wall layers deposited after the transient.

That the shift in microtubule orientation is morphogenetically significant

Fig. 6. (*a*). Longitudinal section through the mid-zone of a nearly mature guard cell of timothy-grass. Note the sublayers in the wall and the criss-crossed, near-axial wall microfibrils and cortical microtubules. ×50000. (*b*). An axial microtubule close to the plasma membrane in a guard cell of timothy-grass. Possible bridging elements are evident (arrows). ×150000.