Planta (1984) 160:289–297

Planta

Organogenesis in *Graptopetalum paraguayense* E. Walther: shifts in orientation of cortical microtubule arrays are associated with periclinal divisions

Jeanne M. Lang Selker* and Paul B. Green
Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA

Abstract. The interior of a new lateral organ, such as a leaf, arises from the products of periclinal divisions of sub-epidermal cells. The biophysical basis of the elongation of such a new axis is transverse (hoop) reinforcement of the cells by cellulose in the primary walls. This structural polarity is associated with transverse alignment of cortical microtubules. We have brought the histological and biophysical views together by showing that the new, periclinal, divisions are a prerequisite for a corresponding change in the orientation of the microtubular array in the daughter cells. Investigation of this relationship required development of criteria for assessing the predominant orientation of a microtubule array in a single section of known orientation. By obtaining information about the predominant orientation of microtubule arrays in the sub-epidermal cells, we were able to study structural polarity shifts which occurred as a detached leaf of *Graptopetalum* produced a new shoot. During organogenesis, the new polarity is seen only in cells which have divided periclinally. Following single periclinal divisions, cells are seen with microtubules in the old or new orientation or in a mixture of different orientations. Cells with more than one orientation of microtubules are probably at intermediate stages in the shift to the new polarity. Among cells which have undergone two consecutive periclinal divisions, the old polarity is no longer seen, all cells having high frequencies of microtubules in the new orientation. Such cells are either polarized in the new direction or nonpolarized. The shifts in polarity of the cells in the interior anticipate the appearance of the first leaf primordia. However, contrary to the expectations from the histological view of organogenesis, these shifts do not dominate the process. Concurrent polarity changes in the epidermis appear at least as important.

Key words: Cellulose microfibril – *Graptopetalum* – Leaf formation – Microtubule – Periclinal division – Polarity.

* *Present address:* Department of Botany, University of Wisconsin, Madison, WI 53706, USA

Introduction

In this study we interrelate two perspectives of plant organogenesis. One view, that of histology, emphasizes that the plant organ is generally an array of parallel cell files, perpetuated by growth and transverse divisions. The other view, that of biophysics, emphasizes that the elongation of an organ has its explanation in the transverse (hoop) reinforcement of the cells by cellulose (see Green 1980). Formation of a new organ requires a change in both the direction of division and the direction of reinforcement. The simplest situation would be if the reinforcement direction shifted at the same time as did the division direction. If this were so, the early periclinal divisions long known to be associated with new organ formation (Sachs 1874, pp. 170–171; Esau 1965, p. 104) would be directly associated with change of reinforcement direction.

There is evidence, however, that a universal correlation between the division direction and other oriented structures of the cell does not exist. In the epidermis of *Graptopetalum*, a division in a new orientation must alter the orientation of the long dimension of a daughter cell relative to the mother cell if it is to be associated with a change in cellulose orientation in that daughter cell (Green and Lang 1981). The new wall from such a division

would be parallel to the long axis of the daughter cell with changed proportions. Also, in *Azolla* roots, orientations of microtubules and divisions are independent (Gunning et al. 1978).

To study the relationship between periclinal divisions in the internal tissue and reinforcement direction, we took advantage of the fact that cellulose orientation correlates with the direction of microtubules in the cell cortex (Hepler and Palevitz 1974; Hardham et al. 1980; Gunning and Hardham 1982). A biophysical polarity, the prospective axis of growth, can be defined as a line normal to the hoops of cellulose and cortical microtubules. Because both wall and cytoplasmic elements throughout the cell are involved, this polarity will be called structural polarity (Fig. 1). One can obtain information about the orientation of the polarity axis by studying profiles of microtubules in sections. At the same time, the orientation of the division giving rise to the cell in question can generally be recognized. Division polarity is defined as perpendicular to the just-formed wall. Thus electron microscopy allows direct examination of the relation between division polarity and structural polarity.

The system used for this study was the formation of new shoots on detached leaves of the succulent *Graptopetalum paraguayense* E. Walther. After detachment, a region of intact tissue with polarities still suitable for the parent leaf begins organogenesis. The region is well defined and is called the residual meristem. It does not begin organogenesis until removed from the parent plant; there is no pre-formed bud (Green and Brooks 1978; Hardham et al. 1981). The epidermis can be observed throughout the formation of the shoot, which takes about 8 d. The timing of the shifts in growth direction is known. In addition, the polarity shifts in the epidermis have been studied (Green and Lang 1981). It was found that shifts in the division direction were a condition for shifts in structural polarity.

The relationship between division direction and structural polarity is considerably more difficult to study in the interior. This is primarily because the interior is three-dimensional while the epidermis is two-dimensional. In the interior, there is greater freedom for both the structural polarity and division direction. Although a 90° shift in both polarities is expected for the development of a new organ, at intermediate stages any orientation relative to the surface, including oblique, appears possible (Green and Lang 1981; this paper). In this study, attention has been restricted to cells whose polarity would be expected to be along one of three

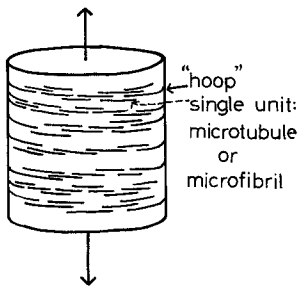

Fig. 1. Model for describing structural polarity of a plant cell. For the purpose of illustration, both cellulose microfibrils and microtubules are considered to form hoops around the cell. The axis of polarity, represented by the double-headed arrow, is normal to the planes of the hoops

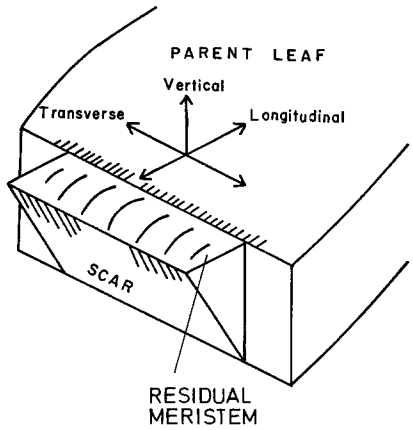

Fig. 2. Diagram after Green and Brooks (1978) defining cell orientations in the *Graptopetalum* residual meristem, as used throughout this paper.The residual meristem is at the base of the leaf, adjacent to the abscission scar. It is shown here partially expanded, with curved lines revealing the surface shape. This is how the meristem would appear from day 2 to 3 (see Fig. 4). Although the meristem is drawn as a separate rectangle, its cell files are actually continuous with those of the rest of the parent leaf, the files merely being narrower in the residual meristem. The orientation of transverse and longitudinal planes for sections and cell walls as defined in this paper are always parallel to the vertical axis. Periclinal divisions are oriented perpendicular to the vertical axis

axes: transverse, longitudinal, or normal to the surface. Since each cell is seen only as a profile in a single section, and since microtubule spatial frequencies are a function of the angle between the microtubule and the section, special arguments have to be made to assess a cell's polarity relative to the three possibilities.

Material and methods

Preparation of plants. Leaves of *Graptopetalum paraguayense* E. Walther were detached from mature plants. Tissue overhanging the residual meristem was trimmed off, and leaves were placed in closed plastic containers in the dark. The time course for growth of the new plant was as described by Green and Brooks (1978). The plants grew slightly faster than those described by Hardham et al. (1980) because they were grown at a somewhat higher temperature (24° C rather than 20° C). The developmental stage of day-5 plants from the study by Hardham et al. (1980) would have been reached on day 4 in plants

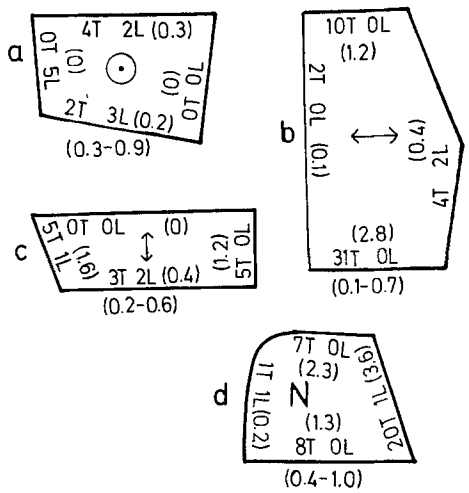

Fig. 3a–d. Data on which polarity assessments for cells in the residual meristem of *Graptopetalum* were based are shown for sample cells from this study. The number of microtubule profiles of each kind (transverse, *T*, or longitudinal, *L*) is shown adjacent to each wall. The single number in parentheses adjacent to each wall is the frequency of transverse profiles (microtubules μm^{-1}) for the adjacent wall. The assigned polarity is drawn in the middle of the cell. The parentheses below each cell enclose a 95% confidence interval for the average frequency of non-array microtubules in an orientation parallel to this section. The confidence interval was computed using Student's t-test for data from a perpendicular section from a plant at the same stage of development. **a** A cell from the transverse section shown in Fig. 4. This is the cell marked with a star in Fig. 4. The frequencies of transverse profiles for all walls are low relative to the average level of non-array microtubules oriented parallel to the plane of this section. Therefore, the cell is assumed to be polarized into the plane of the section. That the cell has a relatively large number of microtubules parallel to the section, as assumed here, is supported by the observation of longitudinal profiles along three of the cell walls. **b** A cell from the longitudinal section shown in Fig. 5 marked with a star. The periclinal walls have high frequencies of transverse profiles relative to the average level of non-array microtubules parallel to the section. These walls both have higher frequencies than the transverse walls. Therefore the cell is assigned an axis of polarity parallel to the longitudinal axis of the parent leaf. **c** A stem cell from the transverse section shown in Fig. 9a (the cell not in a group). The frequency of transverse microtubule profiles along the longitudinal walls is higher than both the average level of non-array microtubules parallel to the section and the frequency of transverse profiles along the periclinal walls. The cell is designated as polarized vertically, the direction correct for the new plant. **d** A cell from the longitudinal section shown in Fig. 9b marked with a single star. The cell is adjacent to the epidermis under the region where the apical meristem forms. Although three of the cell walls have high frequencies of transverse microtubule profiles relative to the average level of non-array microtubules, no single pair of parallel walls has a consistently higher frequency than the other pair. This cell is designated nonpolarized

of the present report. Ink dots were applied to the surface of some plants before fixation to facilitate the orientation of tissue for sectioning, as described by Hardham et al. (1980).

Microscopy. Fixation and preparation of thin sections for electron microscopy were as described by Hardham et al. (1980). Briefly, residual meristems were fixed in 2% glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated in an acetone series, and embedded in Spurr's resin (Spurr 1969). Sections were stained with uranyl acetate and lead citrate. For light microscopy, sections 1 μm in thickness were stained with 1% azure blue II, 1% methylene blue in 1% sodium borate.

Counting of microtubules. For plants at various stages of growth, sections were cut at two perpendicular orientations. Counts were made either from photographic prints or from the image viewed directly in the electron microscope. Prints at a magnification of 34,000× were made from negatives at a magnification of 13,600×. The microscope image on the screen at a magnification of 17,000× was viewed with a binocular microscope to give a final magnification of approx. 170,000×. When microtubules were counted directly from the microscope image, prints of the same section were made at a magnification of 3,600× from negatives at a magnification of 1,440× for the purpose of measuring the dimensions of the cells in which microtubules were counted. The average number of microtubules per μm of wall (defined as the "frequency" of microtubules) was determined for each cell wall. Orientations of sections and divisions are defined as shown diagramically in Fig. 2. These definitions are the same as used previously by Green and Brooks (1978), but different from those used by Hardham et al. (1980).

In longitudinal and transverse sections, profiles of microtubules were counted along the cell walls. Criteria for defining microtubule orientation relative to the section plane are the same as used by Hardham et al. (1980). Briefly, trigonometric relations based on the length of the microtubule profile seen in the section and the average section thickness are used to determine the angle of orientation of the microtubule relative to the plane of the section. The microtubule profiles are divided into three orientation categories: transverse, with the angle of the microtubule to the section plane being 60–90°; oblique (30–60°) and longitudinal (0–30°). Only microtubules within three microtubule diameters from the plasma membrane were tallied.

Judgement of structural polarity. A polarized cylindrical cell is viewed ideally as having its cortical microtubular array as a stack of rings or hoops which lie normal to the cell's axis. If all cells were polarized in exactly that fashion, assessment of polarity in a section would be relatively easy. In fact, however, polarized cells typically show some "stray" microtubules which are not oriented normal to the cell axis. These will be called "non-array" microtubules. The issue of cell polarity rests on the relative preponderance of microtubules in a given direction. If it were possible to section the same cell in two perpendicular planes, this preponderance could be readily recognized. That was not feasible so an indirect procedure was developed.

A cell's polarity had to be judged on the basis of a single profile in a section of known orientation, plus information on the average frequency of non-array microtubules (see Fig. 3 legend) for the tissue at the time in question. All sections were vertical. They were either longitudinal or transverse as diagrammed in Fig. 2. If a four-sided cell profile showed high frequencies of microtubule cross-sections on two opposite sides (frequencies high relative to known non-array frequencies), then the section was apparently intersecting the array effectively and the structural polarity of the cell was in the plane of the section. For example, if the top and bottom profiles were dense with microtubules, the structural polarity would be horizontal, as in Fig. 3b. Double-headed arrows show the polarity. If the left and right profiles were dense, the cell polarity would be vertical, as in Fig. 3c.

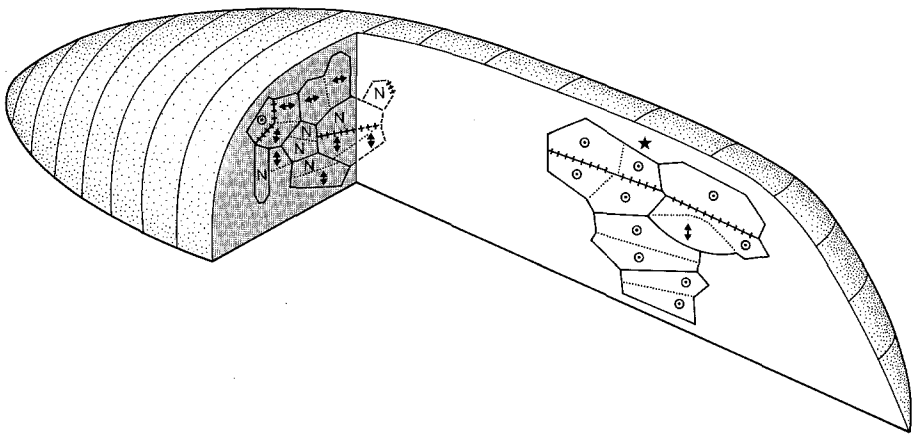

Fig. 4. Summary of assessments for polarity of interior cells in a day-3 *Graptopetalum* residual meristem. The expanded meristem is shown as it would appear after cutting with a longitudinal section (*shaded*) and a transverse section (*unshaded*). In practice, the longitudinal and transverse sections come from different meristems. Cells for which the direction of the axis of polarity was determined are sketched inside the drawing of the meristem. Cells are drawn approximately to scale relative to the overall plant structure. The cells in the transverse section are larger than the cells in the longitudinal section because of the perspective of the drawing. The polarity designation has been drawn inside each cell. Polarity correct for the new plant is represented by a vertical arrow in both the longitudinal and transverse sections. Cross walls laid down in the most recent divisions are drawn with dotted lines (...). The next most recent cross walls are drawn with cross-hatched lines (-l-l-l-). Cross walls from periclinal divisions are approximately horizontal in each section (parallel to the straight line at the base of each section). Transverse divisions are approximately vertical in the longitudinal section, the vertical orientation being parallel to the line between the two sections. Longitudinal divisions are approximately vertical in the transverse section. The cells of the longitudinal section which extend behind the plane of the transverse section have been drawn with dashed lines (---). The cell marked with a star is also seen in Fig. 3a

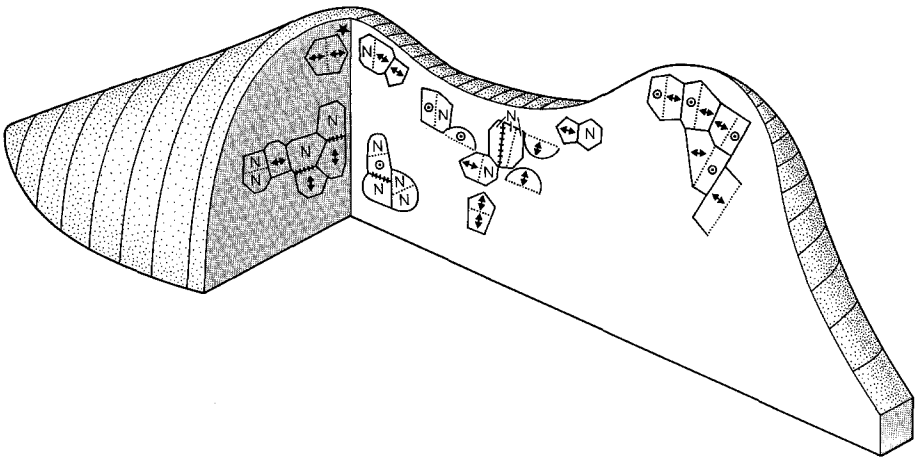

Fig. 5. Summary of assessments for polarity of interior cells in a day-4 residual meristem of *Graptopetalum*. Cells are drawn approximately to scale of the plant. Symbols are as described for Fig. 4. Leaf primordia are of slightly different ages, the smaller one on the left being younger. The cell marked with a star is also seen in Fig. 3b

If however, a four-sided profile showed no obvious preponderance of microtubule sections on opposite sides, the cell was likely to be polarized along an axis running normal to the section. In such a case one would expect to see occasional microtubule profiles in long section (as parallel lines) rather than as small circles. This is shown in Fig. 3a. Unfortunately this is not always a useful criterion because the section is thin relative to the spacing of microtubules. Many sections parallel to the "hoops" of microtubules may contain no microtubules at all. An additional criterion is needed. If the cell is polarized at right angles to the section, the profile should show a low number of microtubules (as low as expected for non-array microtubules), found roughly equally on all four sides of the profile. When this was the case, the cell was scored as having polarity normal to the orientation of the section. This is designated by a circle and dot (Fig. 3a).

If the number of microtubules seen on all sides was high relative to non-array frequencies, or if the microtubule counts were high on two adjacent sides in the profile, then a polarity could not be assigned. Such cells were scored as nonpolarized as in Fig. 3d, although of course slightly aligned arrangements in weakly polarized cells would escape identification by this judgement scheme.

Results

It is possible to observe a transition of cell polarity from that typical of the parent leaf (e.g. horizontal) to that of the newly forming shoot (e.g. vertical). Observations at different stages of growth will be described in detail.

At day zero, microtubules were counted in nine cells (data not shown). Six of the cells were polar-

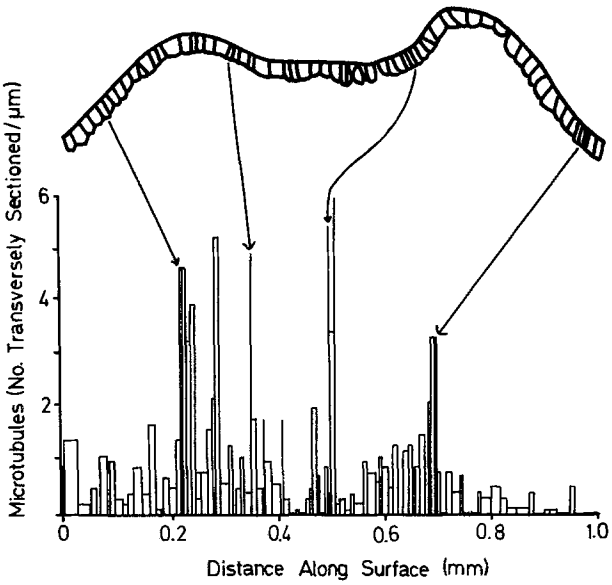

Fig. 6. Graph of frequencies of microtubules in the new orientation for epidermal cells from the transverse section of the residual meristem shown in Fig. 5. In the transverse section, the microtubules in the orientation correct for the new plant are sectioned transversely and thus sampled efficiently. Each bar in the histogram represents data for an individual cell. The drawing above is a tracing of cells from position 0.17 to 0.72 mm on the graph. *Arrows* connect some cells in the tracing to corresponding bars in the graph. The average frequency of microtubules in the old orientation, correct for the epidermis of the parent leaf, was calculated from cells at the leaf tip of the longitudinal section of Fig. 5. This 95% confidence interval (0.5–0.9 microtubules μm^{-1}) serves as a comparison for the frequencies of microtubules in the new orientation, indicating that many of the cells in this section are polarized in the new direction

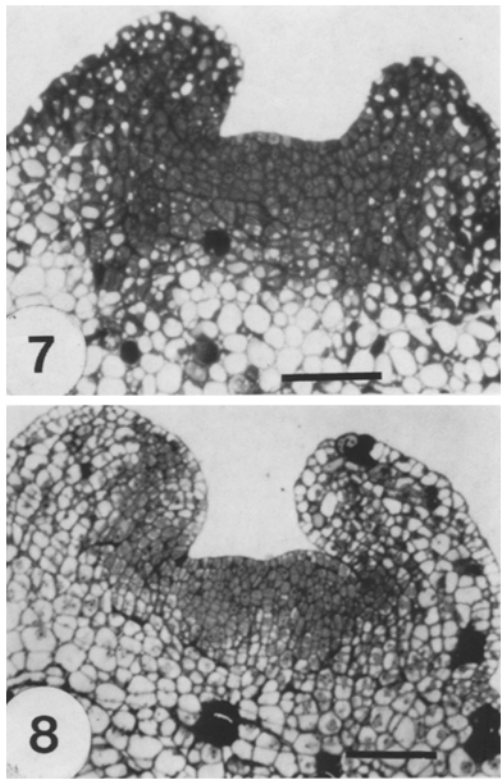

Fig. 7. Day-5 residual meristem of *Graptopetalum* in transverse section. The leaf primordia have enlarged since their initiation a day earlier, but no stem is yet evident. Many recent periclinal crosswalls are seen under the central surface of the meristem, from which the apical meristem will form. Some divisions parallel to these are seen in the leaf primordia. Bar = 100 μm; × 135

Fig. 8. Day-6 residual meristem of *Graptopetalum* in transverse section. The leaves have enlarged dramatically since day 5, and a short stem is now present. At the lower right side of the figure, the notch at the boundary between the leaf and the new stem is seen. Short cell files are seen in the leaves, the stem and below the apical meristem region (to the right of the left leaf). Bar = 100 μm; × 122

ized correctly for the parent leaf, their predicted growth direction being longitudinal. The remaining three cells were nonpolarized. All except one of the most recent divisions had been transverse, the orientation correct for the cell files of the parent leaf. The one exception was a periclinal division where the new wall was perpendicular to the long dimension of the daughter cells. The cells produced by that division were both polarized correctly for the parent leaf.

Between day zero and day 3, some cells in the epidermis (Green and Brooks 1978) and interior (data not shown) divide periclinally. By day 3, the pattern of division in the interior can be seen to be one of alternating periclinal and anticlinal divisions (Green and Lang 1981, their Fig. 23). The new periclinal walls are usually parallel to the long axis of the daughter cell.

Polarity designations for day-3 meristems are summarized in Fig. 4. Some cells have shifted to a polarity correct for the new plant. These cells which have shifted are all among the cells which have recently undergone periclinal divisions. However, many cells which have recently divided periclinally are either polarized in directions other than correct for the new plant or are nonpolarized. This is particularly striking in the transverse section where, in spite of numerous periclinal divisions producing extremely long new walls, all but one of the cells have very low frequencies of microtubules in the new orientation.

Between day 3 and day 4, periclinal divisions continue to alternate with anticlinal divisions in the interior so that no cell files in the new direction are yet evident (Green and Lang 1981, their Fig. 24). Polarity designations for day-4 meristems are summarized in Fig. 5. As at day 3, those cells which have changed to the polarity correct for the

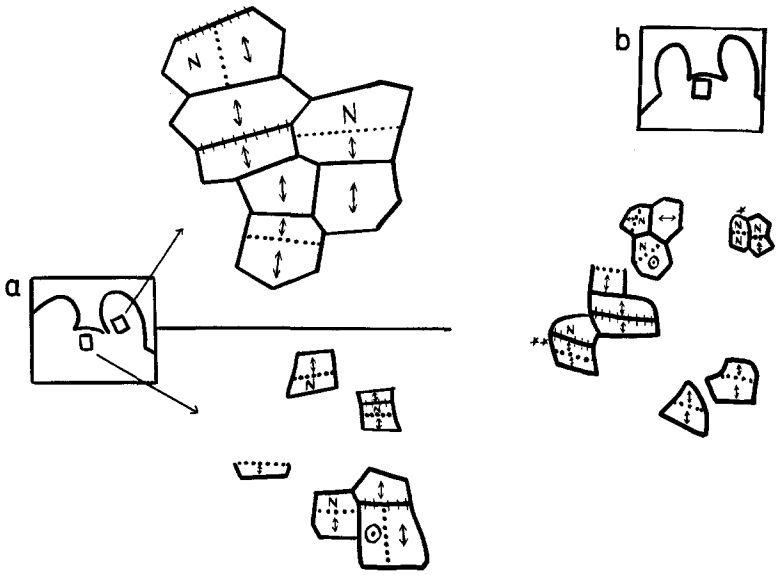

Fig. 9a, b. Summary of assessments for polarity of interior cells in a residual meristem of *Graptopetalum* (day 6). Symbols as in Fig. 5. The outline of the plant as seen in (**a**) transverse and (**b**) longitudinal section appears in the insets. Cells which were analyzed were taken from the regions enclosed in squares. The cell in **b** marked with a single star appears also in Fig. 4. This cell and others drawn level with it are next to epidermal cells. The cell marked with a double star appears in Fig. 10

new plant have all recently divided periclinally. The cells with the new polarity are, as at day 3, not immediately adjacent to the epidermis. They are equally frequent under the future apical meristem region and under the new leaf primordia. Cells under the leaf primordia have elongated dramatically since day 3. This growth has been in the direction correct for the new plant. However, most of these cells still lack the structural polarity which could direct such growth. Conspicuous among these are the cells immediately below the epidermis of the leaf primordia which have dramatically elongated in the new direction. The frequency of microtubules along vertical walls (microtubules in the new orientation) in these cells is consistently low, the mean and 95% confidence interval (Student's t-test) being 0.3 ± 0.1 microtubules per μm. Since the microtubules in the new orientation are efficiently sectioned, there is no reason to doubt that these low counts reflect the true condition of these cells. We also observed the orientation of cellulose microfibrils of some interior cells under the young primordia and found it to be incorrect for the new plant, in agreement with the microtubule patterns (data not shown).

Data for epidermal cells in the transverse section of Fig. 5 are summarized in Fig. 6. Microtubules oriented correctly for the new growth direction are found at high frequencies in the regions of the new leaf primordia. For the younger primordium, the cells with the highest frequencies occur sporadically all across the bulge. For the older primordium, cells with strikingly high frequencies of microtubules in the new orientation occur at the margins of the primordium.

Between days 4 and 5, many interior cells not immediately adjacent to the epidermis divide periclinally (Fig. 7). By day 6, short cell files have appeared in the leaves and in the now conspicuous stem as cells continue to divide in this new direction (Fig. 8). Most cells at this stage have thus divided at least twice in the new direction.

In the center of the stem and in the leaf base at day 6, most of the internal cells observed interior to the first two layers adjacent to the epidermis of the apical meristem are polarized correctly for the new plant (Fig. 9a, b). Regardless of the overall cell polarity, the microtubules in the new orientation in these cells occur at a high frequency, the mean and 95% confidence interval being 2.6 ± 0.4 microtubules per μm. Two of the cells seen in Fig. 9a have the polarity correct for the new plant although the most recent division has not been a periclinal division. However, it is clear in both cases that a previous division had been periclinal.

We observed that the orientation of recently deposited cellulose in cells with the new microtubule polarity is the expected one for control of growth of the new plant and is parallel to the microtubules (Fig. 10).

A summary of the data from all sections from day zero to day 6 appears in Table 1. With the exception of the two cells at day 6, none of the cells with the most recent division in an orientation other than periclinal has the new polarity. A contingency table and χ^2 statistic computed from it (Sokal and Rohlf 1969) allow us to reject the hypothesis that the shift to the new polarity is independent of periclinal divisions (Table 2).

Although the polarity of division direction re-

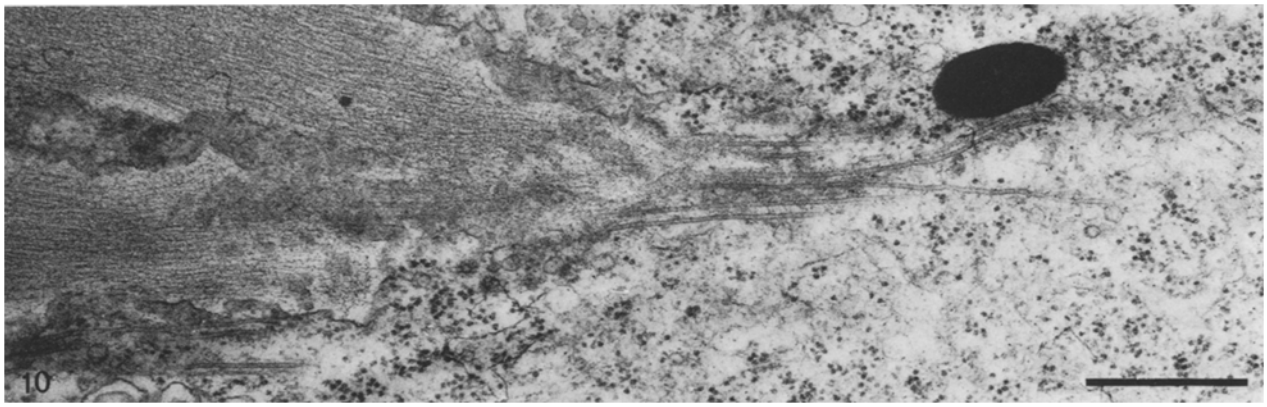

Fig. 10. Region of the cortical cytoplasm and inner wall of the cell next to the double star in Fig. 9b. The section has cut a transverse wall obliquely. The microtubules and microfibrils of cellulose are both oriented correctly for controlling growth of the new plant. Microtubules can be seen exactly parallel to ajacent microfibrils. Bar = 0.5 μm; × 42,500

Table 1. Summary of data from sections of the residual meristem of *Graptopetalum* from day zero to day 6. Sections were either longitudinal (L) or transverse (T). The number of cells polarized correctly for the new plant (↕) and polarized in some other orientation (other) is tabulated for cells which have recently been produced by periclinal divisions and for cells not recently produced by such divisions

Day	Section orientation	Number of cells with new (↕) or other polarity			
		Cells recently produced from periclinal divisions		Cells not recently produced from periclinal divisions	
		↕	Other	↕	Other
0	L	0	1	0	8
3	L	6	6	0	4
3	T	1	6	0	4
4	L	2	4	0	3
4	T	4	6	0	23
5	T	0	3	0	6
6	L	9	4	0	5
6	T	15	5	2	0
Column totals		37	35	2	51
Totals for each division type		72		53	
Total number of cells		125			

Table 2. A contingency table for the data from Table 1. The χ^2 statistic is computed for the table as described in Sokal and Rohlf (1969). Here $\chi^2 = 32.26$. The critical value of χ^2 for $\alpha = 0.01$ is 6.635. Therefore, the shift to the new polarity is not independent of a preceding periclinal divison

Polarity	Cell recently produced from a periclinal division	Cell not recently produced from a periclinal division	Sum
↕	37	2	39
Other	35	51	86
Sum	72	53	125

mains stable in the interior during elongation of the stem and leaves, producing regular cell files, the high level of organization of structural polarity for the interior seen at day 6 does not persist. Fifteen cells were examined in the stem and oldest leaf near the stem-leaf junction in a day-10 plant. Only five were polarized correctly for the new plant. Twelve cells were nonpolarized, and three were polarized perpendicularly to the direction of growth (data not shown). In contrast, all of 65 epidermal cells examined were polarized correctly for the direction of growth shown by the stem.

Discussion

That the interior of a new organ arises in association with new (periclinal) cell divisions is well established (Sachs 1874, pp. 170–171; Esau 1965, p. 104). Association of the new growth direction of the organ with the orientation of the cellulose reinforcement in the primary walls has also been noted (Frey-Wyssling 1976; Green 1980). The aim of the present study was to clarify the rules for cell behavior in setting up both the histological pattern of the new organ and its appropriate hoop reinforcement.

Relationship between a division in the new direction and the structural polarity. A division in the new direction appears to be required for a structural polarity shift. While new organs are being produced (days zero to 5), all cells which show new cell polarity are the immediate products of periclinal divisions. Significantly, certain cells that appear to have elongated along the new axis, but are not products of recent periclinal division, lack the new structural polarity (Fig. 5). This indicates

that the direction of cell extension and the physical position in the new organ do not directly affect the polarity. The cells in question have presumably been stretched by the growth of nearby epidermal cells which have already acquired the new polarity.

A new division direction is also required for new structural polarity in the epidermis of *Graptopetalum* (Green and Lang 1981).

The effect of the new division direction may not be immediate. By the criteria used here, polarization in the new direction is more obvious in the progeny of the second consecutive periclinal division of a given cell. Among cells at stages where the orientation of division direction alternates between periclinal and anticlinal (days 3 to 5), only one third of the cells which have recently divided periclinally have the new polarity. Among cells at day 6, where most cells have undergone two consecutive periclinal divisions, three quarters of the cells have the new polarity. A similar effect of repeated divisions in the new orientation is noted in polarized-light images of outer epidermal walls in *Graptopetalum*. There, groups of four cells derived from two successive divisions in the same orientation frequently show better apparent cellulose alignment than simple pairs of cells (Green and Lang 1981, their Fig. 13). Thus while a polarity shift may start with the first division in a new direction, the effects may not be maximal until after a second such division has taken place. The observation of overlapping arrays of cortical microtubules in different orientations in epidermal cells in regions of polarity shift also indicates that shift to the new polarity is gradual (Hardham et al. 1980).

Apparently the transition in structural polarity involves concurrent presence of microtubules with old and new orientations. In our study, any cell with a fairly high frequency of microtubules in more than one orientation was classified as nonpolarized. Between days 3 and 5, 26 of the cells studied were immediate products of a periclinal division but did not have the new polarity. Sixteen of these cells were nonpolarized. At day 6, eight of the cells had just divided periclinally and lacked the new polarity. All of these cells were nonpolarized. These nonpolarized cells had high frequencies of microtubules in the new orientation, comparable to the cells which had the new polarity; the nonpolarized cells merely had additional microtubules in at least one other orientation. These results are consistent with the view that the nonpolarized state can be the intermediate between old and new polarities. The finding of ten cells which were products of periclinal divisions but which showed the parental polarity could mean that the progressive shift sometimes starts out too slowly for a change to be immediately detected from information in a single section.

One reason for a division in a new direction being insufficient even to *start* a polarity shift was found from examination of the epidermis. There it appeared that the new type of division produced cells with a changed polarity only when the proportions of the daughter cell were also changed. For example, a "broad" mother cell, say six units wide and only four units tall, could divide longitudinally to give two daughter cells, each three units wide and four units tall. These daughter cells would be "tall", hence of new proportions relative to the mother cell. The daughter cells would be of the new polarity. In the interior, all except two of the periclinal divisions probably *were* effective in changing the orientation of the long dimension of the cell. Cells which have recently divided periclinally but do not have their longest dimension parallel to the periclinal wall probably have elongated since the division occurred (for example, the four cells in the longitudinal section in Fig. 5 shown with cross-hatched periclinal walls). The observations in the interior are consistent with the proportion criterion in that all cells which shifted to the new polarity have the expected cell proportions relative to the new periclinal wall. In the two clear cases of periclinal divisions which did not change cell proportions, a cell at day zero and one at day 3, the structural polarity remained parental. The proportion "rule" could help to explain why two cells in the transverse section at day 6, one in the leaf and one in the stem, were found to have retained the new polarity in spite of having recently divided in a direction other than periclinal. At this stage, the cells were becoming extremely broad. It is likely that a vertical division could have occurred in one of these broad cells without changing the cell major dimension. Subsequent elongation, known to be occurring rapidly in this region, could have altered the shape of the daughter cells to become approximately isodiametric, as in the leaf cell, or vertically elongate, as in the stem cell. These two cells were both slightly wider than their adjacent sister cells which did not have the new polarity.

In the root of *Azolla,* structural polarity is not affected by division orientation (Gunning et al. 1978). This apparent counterexample indicates that it is necessary to postulate a hierarchy of rules governing shifts in structural polarity. First, tissues must be in a condition where polarity shifts are allowed. The control may be hormonal or physical.

The root cells of *Azolla* would be an example of cells in a non-permissive condition. Second, when polarity shifts are possible, they may be initiated in the products of a given mitosis when at least two conditions are met: 1) the new cross-wall must be in a new orientation, and 2) the long dimension of the daughter cell must be parallel to the recent cross-wall. This is a more complex picture than a simple strict coupling of division direction to structural polarity.

Relative roles of epidermal and interior cells in initiation of primordia. Many accounts of morphogenesis assume a dominant role for the activity of the cells in the interior (see review by Green 1980). While isolated periclinal divisions have frequently been associated with early events in the production of lateral organs, the total pattern of division and expansion which generates a primordium has received less attention. Lyndon (1982) reexamined leaf initiation in pea through one plastochron. The data related to the orientation of division in both the epidermal and sub-epidermal cells. Serial longitudinal sections allowed him to classify division orientation in three mutually perpendicular directions. He noted that the epidermis retained its longitudinal polarity while division orientation in the interior was temporarily randomized. His observations, along with ours, run counter to the view that histological changes in the sub-epidermal cells dominate organ formation.

Lyndon did not identify a local preponderance of special polarity-shifting divisions in the epidermis, as we have reported for *Graptopetalum* (Green and Lang 1981). If the new organ epidermis consists of files radiating from a pole, then polarity-shifting divisions are inescapable (Lyndon's Fig. 1). Their relative number, however, is a function of leaf shape and histology. The flat pea leaf primordium approximates a two-dimensional triangle compared with the cylindrical form in *Graptopetalum*. In pea, most of the files tend to run strictly parallel to each other, parallel to the altitude of the triangle, rather than radiating from the tip. This indicates that the great bulk of the leaf surface is apparently derived from a bowing out of the epidermis without change in polarity. The abrupt shifts would occur only in the restricted regions of the future leaf margins.

In the present study, involving observations on microtubule arrays in the epidermis and interior of *Graptopetalum*, polarity shifts begin at an early stage in both tissues. If anything, they are completed earlier in the epidermis. The two tissue types appear to follow similar rules and to be of equivalent importance in a three-dimensional reorganization of symmetry.

We thank Fran Thomas for technical assistance with the electron microscope and Peter Ray, Eric Selker and Norman Wessells for helpful discussions. The research was supported by National Science Foundation grant PCM 78-03244,A1,2 to P.B.G.

References

Esau, K. (1965) Plant anatomy, 2nd edn. Wiley, New York

Frey-Wyssling, A. (1976) The plant cell wall. Gebr. Borntraeger, Berlin

Green, P.B. (1980) Organogenesis – a biophysical view. Annu. Rev. Plant Physiol. **31**, 51–82

Green, P.B., Brooks, K.E. (1978) Stem formation from a succulent leaf: its bearing on theories of axiation. Am. J. Bot. **65**, 13–26

Green, P.B., Lang, J.M. (1981) Toward a biophysical theory of organogenesis: birefringence observations on regenerating leaves in the succulent, *Graptopetalum paraguayense* E. Walther. Planta **151**, 413–426

Gunning, B.E.S., Hardham, A.R. (1982) Microtubules. Annu. Rev. Plant Physiol. **33**, 651–698

Gunning, B.E.S., Hardham, A.R., Hughes, J.E. (1978) Evidence for initiation of microtubules in discrete regions of the cell cortex in Azolla root-tip cells, and an hypothesis on the development of cortical arrays of microtubules. Planta **143**, 161–179

Hardham, A.R., Green, P.B., Lang, J.M. (1980) Reorganization of cortical microtubules and cellulose deposition during leaf formation in *Graptopetalum paraguayense*. Planta **149**, 181–195

Hepler, P.K., Palevitz, B.A. (1974) Microtubules and microfilaments. Annu. Rev. Plant Physiol. **25**, 309–362

Lyndon, R.F. (1982) Changes in polarity of growth during leaf initiation in the pea, *Pisum sativum* L. Ann. Bot. **49**, 281–290

Sachs, J. (1874) Lehrbuch der Botanik, 4th edn. Engelmann, Leipzig

Sokal, R.R., Rohlf, F.J. (1969) Biometry; the principles and practice of statistics in biological research. Freeman, San Francisco

Spurr, A.R. (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**, 31–43

Received 20 April; accepted 6 July 1983