Polarized cytokinesis in vacuolate cells of *Arabidopsis*

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The view of plant-cell cytokinesis commonly depicted in textbooks is of a symmetrical process, with the phragmoplast initiating in the center of the cell and growing outward to the parental cell membrane. In contrast to this picture, we observe that cell-plate development in *Arabidopsis* shoot cells is highly polarized along the plane of division. Three-dimensional live-cell imaging reveals that the mitotic spindle and phragmoplast are laterally displaced, and that the growing cell plate anchors on one side of the cell at an early stage of cytokinesis. Growth of phragmoplast across the cell creates a new partition in its wake, giving the visual effect of a curtain being pulled across the cell. Throughout this process, the advancing front of the phragmoplast is in intimate contact with the parental wall, suggesting that short-range interactions between the phragmoplast and plasma membrane may play important roles in guiding the cell plate throughout much of its development. Polarized cytokinesis was observed in a wide variety of vacuolate shoot cells and in some small root cells, implying that it is not solely a function of cell size. This mode of cytokinesis may provide a mechanically robust mechanism for cell-plate formation in large cells and suggests a simple explanation for the occurrence of cell wall stubs observed upon drug treatment or in cytokinetic mutants.

One of the fundamental tasks of cell function is cytokinesis, the division of one cell into two or more daughters. The cells of animals and many bacteria accomplish this task through mechanisms that progressively constrict the cortex of the cell (1, 2). Higher plant cells differ markedly from these systems in the geometry and mechanisms of cytokinesis. Rather than constricting at the periphery, plant cells build their partitions from the interior outwards by using a specialized structure called the cell plate.

Since the time that Sinnot and Bloch published their elegant descriptions of higher plant-cell cytokinesis in 1940–41 (3, 4), the cell's center has been highlighted as the location of cell-plate initiation and subsequent growth. Their observations, together with those of many subsequent authors (reviewed in refs. 5–8), have produced a well established picture of higher plant-cell cytokinesis in which the preprophase nucleus migrates to the cell's center, where it is suspended by thick transvacuolar strands of cytosol that connect the nucleus to the future site of division. This arrangement of cytosol was termed the phragmosome by Sinnot and Bloch (3). At late anaphase, formation of the phragmoplast begins, creating a specialized environment for development of the cell plate. An early marker of the phragmoplast is a unique array of short parallel microtubules that form a raft facing the spindle poles (9). Actin filaments, Golgi-derived vesicles, the endoplasmic reticulum, and a growing list of proteins (reviewed in ref. 10) accumulate in the phragmoplast and facilitate growth of the cell plate and new cell wall. High-resolution fast-freeze fixation methods have revealed that the vesicles at the midplane of the phragmoplast fuse and progressively coalesce in a series of events to form tubulo-vesicular cisternea, a fenestrated plate, and finally a solid cell plate (11). The fusion of vesicles to the edge of the growing cell plate creates a centrifugally expanding disk that grows out to the cell cortex where it fuses with the plasma membrane.

Many questions in the field of plant cytokinesis relate to how the cell plate (i.e., division plane) is oriented, how its growth is controlled, and how its fusion with the parental membrane is regulated. Although relatively little is known about any of these mechanisms, distinct modes of regulated membrane fusion have been posited to account for separately controlled vesicle fusion, cell-plate growth, and its subsequent fusion with the plasma membrane (10, 12, 13). The division site is predicted by the preprophase band (PPB) (14, 15), a plant-specific microtubular array that may be associated with the deposition of guidance cues that direct the cell plate to the plasma membrane (16–19). These mechanisms act together to ensure that the cell divides on the selected plane, an important determinant of developmental pattern (20).

The immense variation in plant cell size and, particularly, variation in vacuolar volume creates an additional set of questions. For example, in large vacuolated cells, how is the delicate edge of the cell plate accurately guided, despite the vigorous motions of cytoplasmic streaming? How does the cell plate push its way through vacuoles that in many shoot cells can be dozens of microns wide? Being interested in these and related questions, we initiated a series of live-cell imaging experiments characterizing cytokinesis in vacuolated cells of *Arabidopsis* by using a combination of targeted green fluorescent protein (GFP) markers and confocal microscopy. These methods allowed us to extend live-cell observation of cytokinesis to many cell types that have been difficult to image in intact organisms, in particular, the epidermal and cortical cells of the hypocotyl, petiole, and leaf blade. To our surprise, we observed a pattern of cytokinesis that is markedly different from the canonical descriptions of a cell plate that traverses the great distance of the vacuole suspended centrally and symmetrically inside the cell without contact with the plasma membrane and wall. Three-dimensional imaging of vacuolated *Arabidopsis* cells reveals a noncentralized and highly polarized mode of cell-plate development. This mode of cytokinesis, which we term "polarized cytokinesis," was observed in all vacuolated cells of the shoot examined and in some small root cells, implying that it is not solely a function of cell size. Because the geometry of cytokinesis is a function of its underlying molecular mechanisms, polarized cytokinesis has several interesting implications for our understanding and further investigation of the mechanisms of plant-cell cytokinesis.

Materials and Methods

Targeted GFP Lines Used for Imaging. Most transgenic lines used for imaging in this paper have been described previously (21). Experiments were performed on T2 or T3 seed derived from the primary transgenic plants (T1) isolated in our original marker screen (21).

Enhanced Green Fluorescent Protein (EGFP). Cytosol and nucleoplasm were illuminated by using soluble EGFP, with plants transformed with the pEGAD EGFP expression vector (21).

Abbreviations: GFP, green fluorescent protein; EGFP, enhanced green fluorescent protein.

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Shoot cells undergoing cytokinesis are readily identified by the aggregation of cytosol in and around the phragmoplast.

LE8. This marker is a cytosolic (nuclear excluded) protein that accumulates in late prophase nuclei, allowing easy identification of cells before cytokinesis (21). This transgenic line expresses an in-frame fusion protein between EGFP and GF14A, a14–3-3 protein (21).

Q4. This marker localizes to the endoplasmic reticulum (ER) membrane and is a fusion between GFP and another protein with a predicted carboxy-terminal transmembrane domain (GenBank accession no. AAB71445; see ref. 21).

EGFP::TUB1. This marker is an N-terminal fusion of EGFP to a cDNA for *Arabidopsis* β-1 tubulin (GenBank accession no. M20405). See supporting information for further details on the construction of GFP::TUB1.

Plant Growth and Mounting. *Arabidopsis* seeds were stratified at 4°C for 2–4 days and then germinated on agar-solidified medium containing Marishige Skoog salts buffered to pH 6.0 with 10 mM Mes·KOH (MS 6.0). Seedlings (2–4 day old) were mounted on coverslips in water or buffered MS 6.0 medium. Seedlings were immobilized with a second coverglass held in position with silicon vacuum grease. In some cases, seedlings were mounted in a thin layer of solidified 4% low-melt agarose and overlaid with 1 mm of water or buffered MS 6.0 medium.

Microscopy and Image Analysis. Confocal imaging was performed by using a Bio-Rad MRC1024 laser scanning confocal head mounted on a Nikon Diaphot 200 inverted microscope equipped with a 60× Nikon Plan Apo 1.2 n.a. water-immersion objective (Technical Instruments, San Francisco). EGFP was excited at 488 nm and emitted fluorescence was collected through a 525/30 band pass filter. Images were analyzed, and three dimensional reconstructions of image stacks were generated with LASERSHARP software (Bio-Rad), or NIH IMAGE (http://rsb.info.nih.gov/nih-image/). Four dimensional data sets were analyzed with either LASERSHARP or 4D TURNAROUND (http://www.loci.wisc.edu/4d/java/4d turnaround java.html).

Results and Discussion

Live-Cell Confocal Imaging of Cytokinesis in Vacuolated Cells Using GFP. The high mitotic indices of meristematic cells have made them favored specimens for studies of plant cytokinesis. To investigate the impact of the large central vacuole on cytokinesis, we examined a variety of targeted and untargeted GFP marker lines to identify structural or dynamic markers of cytokinesis in shoot tissue. We found that untargeted EGFP (a cytosolic label) enabled identification of early stages of cytokinesis because it labeled a distinctive arrangement of nuclei and cytosol in cytokinetic cells. Two daughter nuclei are closely positioned to either side of a thin wall of cytoplasm that is often bisected by a zone of cytoplasmic exclusion, presumably corresponding to the dense array of vesicles and membranes of the new cell plate (Fig. 1*A*). The phragmoplast is visible as a dense aggregation of cytoplasm at the edge of this wall. This thick band of cytoplasm possesses an arrowhead-like appearance in cross section (Fig. 1 *A* and *D*), and when viewed side-on, it is relatively uniform in thickness, with occasional tubule-like projections (Fig. 1*D*). Intense labeling of this band of cytosol by a GFP fusion to *Arabidopsis* β-tubulin confirmed its identity as the phragmoplast (see Fig. 3). In most cells, cytosolic connections between the cell nuclei and the phragmoplast were apparent (Fig. 1*A*). Thus, the conspicuous aggregation of cytoplasm around the phragmoplast enables ready identification of shoot cells undergoing cytokinesis. Similar aggregations of cytosol were observed by differential interference contrast (DIC) microscopy in untransformed transgenic lines, suggesting that they are not a peculiarity of the EGFP marker line used (D.W.E., unpublished observations).

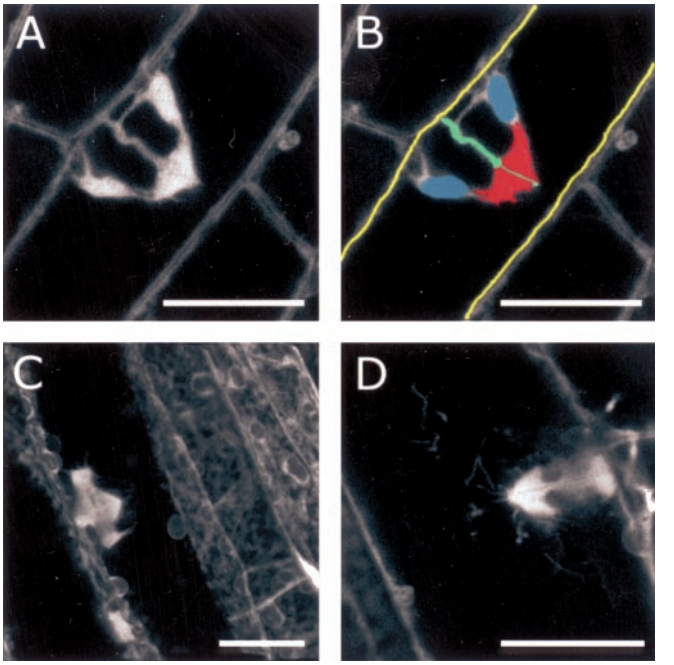

Fig. 1. Cytokinetic structures visualized in living cells by confocal imaging of cytosolic EGFP. (*A*) Confocal section through a highly polarized cytokinetic apparatus in an epidermal cell of the cotyledon petiole. (*B*) Color overlays identify key structures seen in *A*: blue, nucleus; green, cell plate/midline of phragmoplast; red, phragmoplast; yellow, cell cortex. (*C*) Confocal reconstruction of a mitotic structure located at the edge of an epidermal cell of the upper hypocotyl. The daughter nuclei are suspended at either end of a spindle-shaped arrangement of cytosol. (*D*) A phragmoplast at the edge of a hypocotyl cell exhibiting numerous tubule-like projections into the vacuole. (Bars = 10 microns.)

Polarized Growth of the Cell Plate in Vacuolate Cells. Two features of the cytokinetic structures identified stood out as unusual. First, they were displaced to the edge of the cell and not suspended in the cell's center (Fig. 1). Second, the phragmoplast was typically crescent-shaped and not shaped like a complete ring, as would be expected for a centrally located cell plate (Fig. 2 and Fig. 3). To ascertain if these peculiarities were consequences of aborted or aberrant cell division events, we tracked numerous polarized phragmoplasts 20–30 min after their identification. In each case (n = 30), the crescent-shaped band resolved into a complete new cell wall (Fig. 2) with apparently normal morphology, suggesting that the polar cytokinetic structures are not consequences of aborted or aberrant cell-division events.

We subjected several cells to time-lapse imaging at a single image plane after the formation of phragmoplasts throughout cytokinesis in vacuolated shoot cells. These image series revealed a highly polarized and directional pattern of cell-plate development, starting at one side of the cell body and progressing smoothly to the opposite side, taking approximately 50 min to traverse the width of typical cells (12–15 microns; Fig. 4 and Movie 3, which is published as supporting information at the PNAS web site, www.pnas.org).

Concerned that we might be selectively identifying a subset of cytokinetic events (because of the conspicuous nature of the polarized phragmoplasts), we imaged cells in stages of the cell cycle preceding phragmoplast formation by using the localiza-

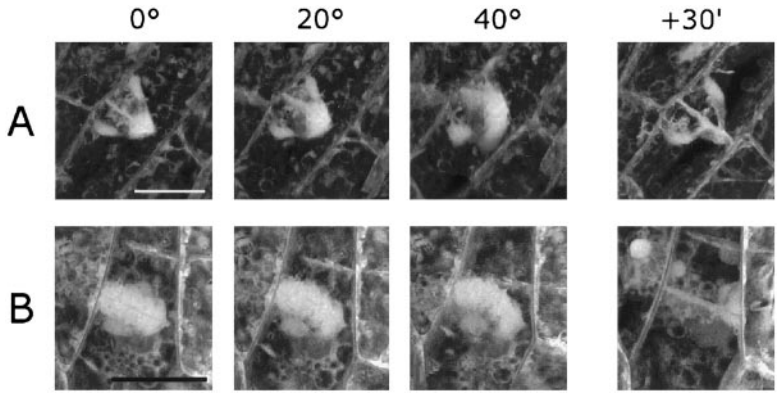

Fig. 2. Cortically anchored cell-plate development. Three-dimensional images of polarized and anchored cell plates in epidermal cells of the upper hypocotyl (*A*) and of the cotyledon petiole (*B*). In each series, panels 1–3 show computer reconstructions of confocal sections projected at 0°, 20°, and 40° from the optical axis of the microscope. The phragmoplast is visible as a crescent-shaped aggregation of cytoplasm at the growing edge of the cell plate. Panel 4 is the same cell approximately 30 min later, showing the completed new cell wall. The cell in *A* is expressing cytosolic EGFP; the cell in *B* is expressing GFP marker LE8, a cytosolic protein that translocates into nuclei during prophase (21). These three-dimensional rotations can be seen in Movies 2A and 4B, which are published as supporting information on the PNAS web site. (Bars = 10 microns.)

tion tag LE8; a marker was recovered in a screen of random GFP::cDNA fusion proteins (21). LE8 is a cytosolic fusion protein that translocates into prophase nuclei (before nuclear envelope breakdown) and, over the course of mitosis, gradually dissipates from the daughter nuclei (21). LE8 is thus a convenient marker for locating cells in late prophase. Because condensed chromatin excludes LE8 label, chromosomes are visible in negative contrast, allowing clear identification of metaphase, anaphase, and telophase stages of mitosis (Fig. 4 *1–3*).

LE8 was used to identify and image prophase cells by using three-dimensional time lapse imaging. In all cells imaged, the nucleus was displaced to the cell periphery at prophase ($n = 12$). After nuclear reassembly, phragmoplast formation was symmetric with respect to the two chromosomal poles and developing daughter nuclei (Fig. 4 *4*). However, the phragmoplast was anchored on the cell periphery adjacent to the position of the mitotic spindle shortly after its appearance (Fig. 4 *5*). After this initial contact, the leading edge of the phragmoplast traveled along the cell periphery in two opposite directions, forming two zones of advancing interaction between the phragmoplast and the parental cell cortex (Movie 4, which is published as supporting information on the PNAS web site). As these fronts advanced, the entire phragmoplast assumed a crescent-shaped structure (Figs. 2 and 3; also see Movies 2 *A* and *B* and Movies 4 *A–D*, which are published as supporting information on the PNAS web site). Progression of the phragmoplast across the cell created a new partition in its wake, producing the visual effect of a curtain being pulled across the cell.

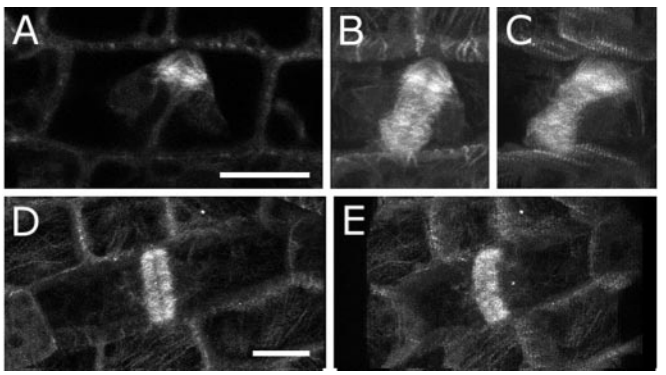

Fig. 3. Polarized phragmoplasts visualized with GFP::TUB1. The phragmoplast microtubule array is seen as a crescent-shaped concentration of label at the edge of polarized cytokinetic structures. (*A–C*) Hypocotyl cell seen as a single confocal section (*A*), a 0° projection (*B*), and a 45° projection (*C*). (*D–E*) Petiole cell seen in 0° (*D*) and 40° (*E*) projections. (Bars = 10 microns.)

Polarized Cell-Plate Development in Root Meristem Cells. Because the pattern of cell-plate formation we observed in shoot cells differed from canonical descriptions for meristematic cells, it seemed plausible that there might be a distinct mode of cytokinesis operating in vacuolate cells of the shoot. To address this question, we examined cytokinesis in cells of the root meristem. Cytosolic GFP was less effective for imaging cytokinesis in root cells because they are densely cytosoplasmic and less vacuolated than most shoot cells. A previously identified endoplasmic reticulum membrane marker, Q4 (21), was found to accumulate on the cell plate and proved useful for imaging cell-plate development and cytokinesis in root cells.

Cells in late prophase or early metaphase were identified by breakdown of the nuclear envelope (Fig. 5, see *1* and *2*) by using the Q4 marker. As cells progressed through telophase, the nascent cell plate accumulated equidistantly between the reforming daughter nuclei (on a focal plane in the center of both nuclei). The cell plate was observed to grow toward both lateral walls until it contacted those walls. Growth of the cell plate was frequently symmetric with respect to the cell's center (Fig. 5, *4–6*); however, in a subset of cells (2 of 8), growth of the cell plate displayed clear polarity, similar to that observed in vacuolate cells of the shoot (Fig. 4). In these cells, the daughter nuclei were displaced laterally, and the cell plate grew in a polar manner after apparently contacting the cell cortex at an early stage (Fig. 5, *1–3*, and see supporting information). Thus, polarized growth of the cell plate is not an exclusive property of shoot cells. This interpretation was confirmed with observations of GFP::TUB1 (data not shown) and of an independent GFP marker, N6, a chromosomal marker that additionally illuminates the cytosolic aggregation of the phragmoplast (see supporting information). Three of six dividing root cells expressing N6 showed polarized cell-plate growth (data not shown).

Nonrandom Orientation of Cell-Plate Development in the Epidermis. One question raised by polarized cell-plate growth is whether the position of initial contact with cell cortex and the direction of plate growth are nonrandom. To ask whether there is a bias in the direction of phragmoplast polarity, we surveyed 52 early phragmoplasts in shoot epidermal cells and asked whether the

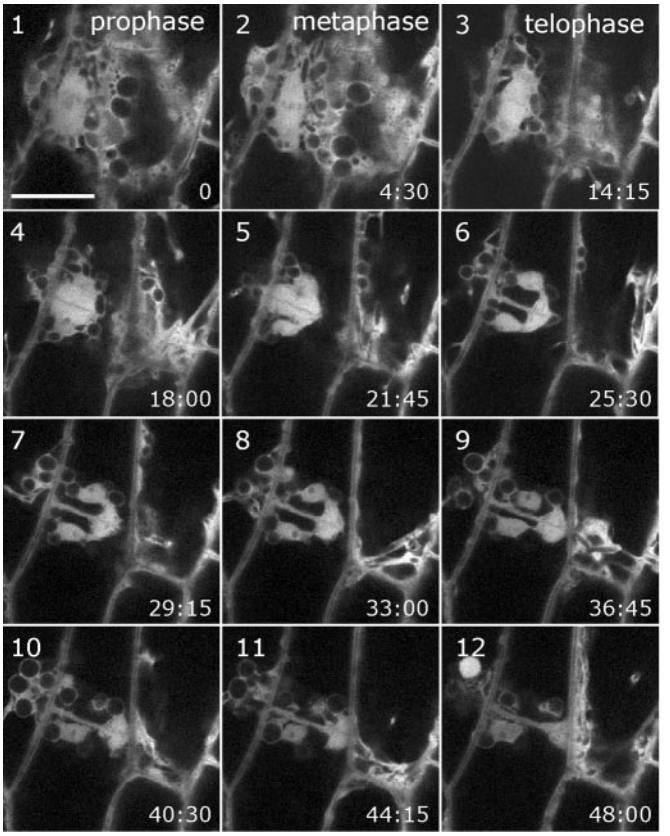

Fig. 4. Polarized cell-plate development seen in time series. A single confocal plane of a cell expressing LE8 (also seen in Fig. 2*B*) was imaged at 50-s intervals. The cell plate contacts the parental membrane at one side of the cell cortex, then grows across the cell to the opposite lateral wall. The elapsed time of each image in min and s is shown in the lower right corner of the image. For a three-dimensional view of this image series, see supporting information. (Bar = 10 microns.)

position of the phragmoplast was consistent with initial cortical contact at the epidermal face of the cell or with contact with any other point around the circumference of the cell. No early contact events at the epidermal face were observed; however, determinations could not be made clearly in eight of the cells surveyed. In the 44 cells where the position of initial cortical contact could be clearly identified, it occurred at points where the dividing cell contacted other cells, suggesting that this event may be sensitive to information generated by cell–cell contact.

Three-Dimensional Data Reveals Polarity of Cytokinesis. In some contexts, polarized phragmoplasts may appear symmetric because of an observational artifact relating to the angle of observation. To illustrate this point, we show different angle-reconstructions generated from three dimensional data sets of a phragmoplast in an epidermal cell (Fig. 6). When viewed in the periclinal plane of the epidermis, the phragmoplast is centered in the cell and thus appears to grow in a symmetric fashion toward the lateral cell walls (Fig. 6*A*). However, this perspective is deceptive. Computer-aided resectioning of the same confocal image data on the periclinal plane reveals the phragmoplast is highly polarized, being anchored to the cortical face of the cell and growing directly toward the epidermal face of the cell (Fig. 6*B*). Thus, three-dimensional data, easily acquired by using GFP markers and confocal microscopy, is necessary to accurately determine the polarity of cytokinesis, and a lack thereof may have obscured descriptions of polarized cytokinesis in the past.

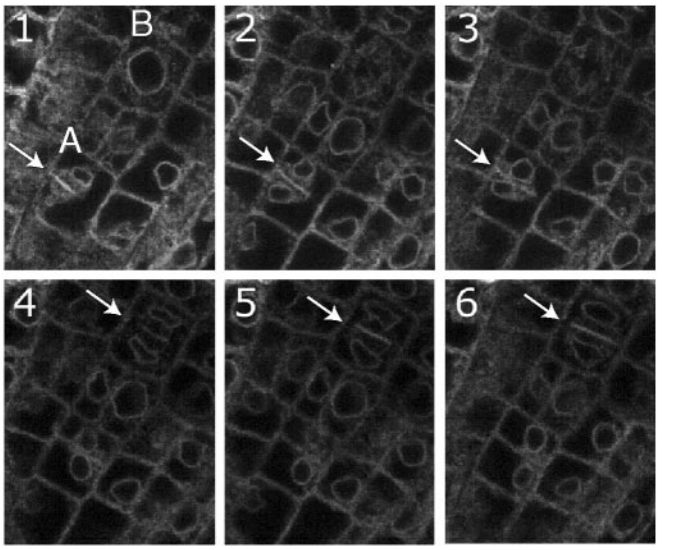

Fig. 5. Root-cell cytokinesis can be polarized. A time-lapse series of a single confocal plane in the cortical-cell layer of the root meristem. Cell A shows a clearly polarized mode of cytokinesis, whereas cell B shows a more symmetrical pattern on the plane of focus. (*1*) The plate in cell A has contacted the cell cortex at one lateral wall. (*2–4*) It grows to the opposite wall. The nuclear envelope of cell B has not yet broken down in *1*, as shown by the bright ring of label around the nucleus. (*2*) Taken 120 s later, the nuclear envelope has broken down, and condensing chromatin is seen as dark areas excluding the GFP label. (*5*) Newly formed daughter nuclei are visible, with a nascent cell plate midway between them. Time intervals are 120 s. (Bar = 10 microns.)

Is Polarized Cytokinesis Peculiar to *Arabidopsis*? Given the predominance of polarized cytokinesis we have observed in *Arabidopsis*, why has polarity not been reflected in standard models of plant-cell cytokinesis? One possibility is that *Arabidopsis* may be an exception among plants; however, examples of polarized cytokinesis in other species have been described. For example, a careful reading of Sinnot and Bloch's classic observations reveals a description of polarized cytokinesis in *Polygonum sachlinense* cells that were stimulated to divide by wounding (4). These events were considered a minor variation on a predominant pattern of symmetric division

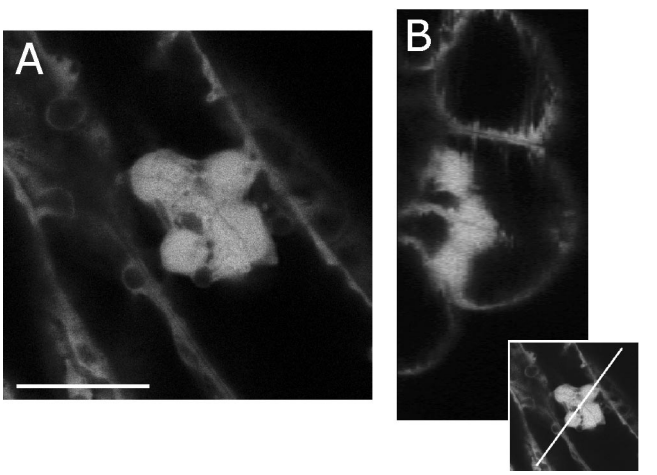

Fig. 6. Detection of cell-plate polarity is influenced by the angle of view. (*A*) A periclinal confocal section of a cell expressing cytosolic EGFP shows a centered and symmetrical phragmoplast. (*B*) Auticlinal section taken along the plane shown in *Inset*. The cell plate is anchored at the cortical face of the cell, and the phragmoplast is advancing directionally to the epidermal cell wall. (Bar = 10 microns.)

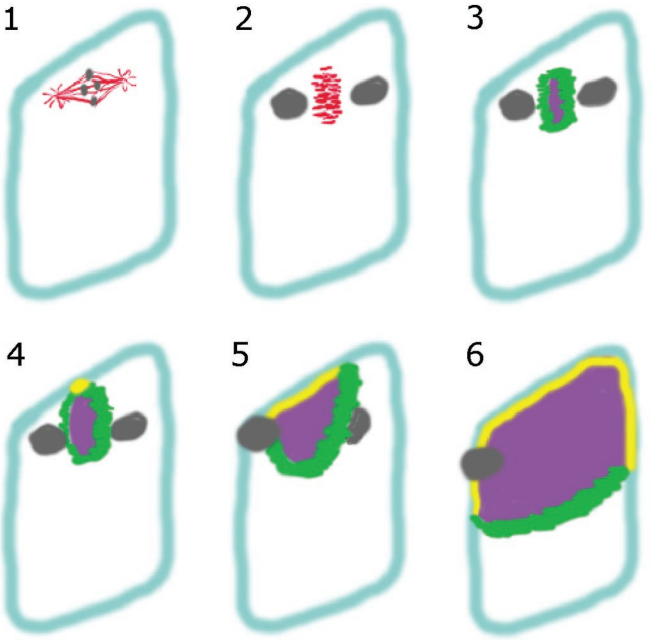

Fig. 7. Schematic summary of cytokinetic events observed in vacuolate *Arabidopsis* cells. The blue line represents the future site of cell-plate insertion marked by presumptive cortical cues. (*1*) The mitotic spindle, shown in red, is adjacent to the cortex of the cell and is positioned next to the future site of cell-plate insertion. (*2*) After anaphase, the phragmoplast microtubule raft begins to form midway between the poles of the mitotic spindle. The phragmoplast remains adjacent to the cortex of the cell. (*3*) Initial elaboration of the phragmoplast (green) and the coalescing cell plate (purple) is symmetrical and grows by radial expansion. (*4*) Contact of the expanding cell plate occurs at a discrete point along the cortex of the cell, where it makes contact with the parental plasma membrane (yellow). (*5–6*) Two zones of contact between the cell plate and the cortex advance in opposite directions along the marked cortex, building the cell plate in a polarized, directional fashion across the cell body.

(4). Several authors since then have described polarized cytokinesis in non-*Brassica* species (22–24), with particularly detailed studies by Venverloo and Libbenga (25) and by Collings and Emons (26). However, these studies were carried out in callus (23) or tissue culture cells (22, 24–26) and were interpreted as being either exceptions to a more common pattern of division or as aberrant. Our observations suggest that polarized cytokinesis is the predominant mode of cell division in vacuolate shoot epidermal and cortical cells during normal *Arabidopsis* development.

Two Phases of Cell-Plate Development? Our observations suggest that there may be two distinct phases of cell-plate growth (Fig. 7). First, there is the initial coalescence of the cell plate between the daughter nuclei and early growth, as described in the standard model and with unprecedented ultrastructural detail by Samuels *et al.* (11). During this early phase of cell-plate development, the phragmoplast and cell plate are radially symmetric. The beginning of the second phase is marked by the initial contact of the growing plate with the cell cortex. This contact occurs at an early stage of cell-plate formation in a large cell and may be associated with fusion of the cell plate to the parental plasma membrane. This possibility is supported by observations of a membrane impermeant dye labeling the growing cell plate, suggesting apoplastic continuity of the parental wall with the cell plate (see Fig. 8, which is published as supporting information on the PNAS web site). After this anchoring event, two distinct zones of intimate contact of the cell plate and the cortex are established. These zones propagate in opposite directions around the perimeter of the cell, connected together by the free front of the phragmoplast. The cell plate is built up as a plane extending from the site of initial cortical contact to the free front of the phragmoplast. The rate of progress of the interaction zones is coordinated with the advance of the phragmoplast front. As the two interaction zones reach the opposite side of the cell from which they started, they meet, resolve, and complete cytokinesis (Fig. 7).

Mechanistic Implications of Polarized Cytokinesis. The geometry of cytokinesis is related to its underlying molecular mechanisms; consequently, our observations have important mechanistic implications. The means by which the cell plate is guided to sites of cortical contact has long been recognized as an issue of central importance to insuring the correct placement of the new cell wall. In the classic centralized model of cytokinesis, the cell plate is a centrally suspended disk and, as a result, it is necessary to hypothesize long-range interactions with the cortex to carry out alignment and guidance functions throughout cell-plate development (7, 27). A popular hypothesis is that the cell plate is guided by tension created by cytoskeletal elements, particularly actin, anchored at the former site of the PPB (7, 24, 28, 29). We have shown that direct contact with the cell cortex occurs at an early stage of phragmoplast development, and that this contact is elaborated and maintained throughout the remainder of cell-plate formation. These observations suggest an important role for short-range interactions at the cell cortex for guiding the alignment of the new cell plate with the site of cortical fusion. A candidate for a short-range interaction mechanism is direct interaction among the class of vesicle-trafficking proteins known as soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors, proteins that have been shown by biochemical and genetic studies to play an important role in cell-plate development (12, 30–32). In addition, the localization of actin to the phragmoplast may contribute mechanical forces for growth of the cell plate. Although the most obvious sign of direct interaction with the cell cortex occurs when the cell plate fuses at the initial contact site, the lateral placement of the nucleus and the spindle before this fusion event suggests that cortical interactions also may act at earlier times.

One of the mechanical puzzles of plant-cell cytokinesis is how a large, highly vacuolate and actively streaming cell supports the stable assembly and growth of a delicate cell plate suspended by cytosolic strands in the center of a displaced vacuole. Anchoring the cell plate by attachment to the cortex at early stages and throughout its growth may provide a mechanically robust means for building a wall in a large, turbulent cell. Cortical attachment also may be particularly important in later stages of cell-plate development—rather than having a large area of free edges, an anchored, polar-cell plate possesses only a small portion of unanchored membranes. This arrangement may provide protection from accidental displacement of the edge of the cell plate by cytosolic buffeting or external disturbance.

Our observations also shed light on the frequent observations of cell-wall "stubs" (23, 33–36) found after treatments with agents that disrupt cytokinesis, such as caffeine (5, 33) and in a variety of mutants defective in cytokinesis (30, 37–39). These stubs have sometimes been interpreted as defective cell plates that initiated but failed to grow and, as a result, anchor stochastically on one of the parental walls (30, 37). Wall stubs also have been explained as vestiges of a primitive cleavage mechanism (33) or as aberrant varieties of wall formation (17, 23, 35). Our observations suggest the simpler explanation that at least some wall stubs may result from normal polar cytokinesis aborted at early stages.

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