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A Small, Novel Protein Highly Conserved in Plants and Animals Promotes the Polarized Growth and Division of Maize Leaf Epidermal Cells

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Summary

Plant cell shapes are defined by their surrounding walls, but microtubules and F-actin both play critical roles in cell morphogenesis by guiding the deposition of wall materials in expanding cells [1–7]. Leaf epidermal cells have lobed shapes, which are thought to arise through a microtubule-dependent pattern of locally polarized growth [8–13]. We have isolated a recessive mutation, *brk1*, which blocks the formation of epidermal cell lobes in the maize leaf. Mutant epidermal cells expand to the same extent as wild-type cells but fail to establish polar growth sites from which lobes arise. In expanding *brk1* epidermal cells, microtubule organization differs little from that in wild-type, but localized enrichments of cortical F-actin seen at the tips of emerging lobes in wild-type cells fail to form. These observations suggest a critical role for F-actin in lobe formation and together with additional effects of *brk1* on the morphogenesis of stomata and hairs suggest that *Brk1* promotes multiple, actin-dependent cell polarization events in the developing leaf epidermis. The *Brk1* gene encodes a novel, 8 kD protein that is highly conserved in plants and animals, suggesting that BRK1-related proteins may function in actin-dependent aspects of cell polarization in a wide spectrum of eukaryotic organisms.

Results and Discussion

brick1 Disrupts Multiple Aspects of Epidermal Cell Morphogenesis

The wild-type maize leaf epidermis is composed of linear files of cells with distinct shapes (Figure 1A). Pavement cells have lobes along their lateral margins, which interlock with those of neighboring cells. Stomatal complexes are composed of two elongated guard cells flanked by two triangular-shaped subsidiary cells (white arrowheads, Figure 1A). Short prickle hairs (white arrows, Figure 1C) and much longer macrohairs (black arrow, Figure 1C) are elongated, with finely pointed ends. We identified a recessive mutation, *brick1* (*brk1*), that completely blocks the formation of epidermal cell lobes (Figure 1B). In addition, *brk1* stomatal subsidiary cells are often abnormal (white arrowheads, Figure 1B), and both prickle hairs and macrohairs are shorter and blunter in *brk1* leaves compared to wild-type (white and black arrows, Figure 1D). In the internal tissue layers of the leaf, mesophyll cells also have lobed shapes. However, examination of leaf cross-sections and isolated mesophyll cells from *brk1* and wild-type leaves revealed no differences in the shapes of mesophyll or other cell types in internal tissue layers (data not shown). Thus, *Brk1* is not required universally for the formation of lobes but rather for lobe formation along with certain other aspects of epidermal cell morphogenesis.

In wild-type leaves, lobe formation is initiated immediately following the completion of cell division and gradually becomes more pronounced as cells subsequently expand 3- to 4-fold to reach their final sizes (Figure 2A). Thus, expanding wild-type pavement cells appear to combine diffuse growth increasing overall size with locally polarized growth occurring at multiple sites along the cell margin to form lobes. Prior to the stage at which lobe formation is initiated in wild-type leaves, no difference is observed in the overall size or shape of *brk1* versus wild-type pavement cells. Subsequently, *brk1* mutant epidermal cells expand to the same extent as wild-type cells but without forming lobes at the same time (Figure 2B). Thus, *brk1* does not cause an overall arrest in growth or development but affects a specific aspect of epidermal pavement cell morphogenesis.

The Failure of Lobes to Form in *brick1* Epidermal Cells Is Associated with Loss of Localized Cortical F-Actin Enrichments

Prior studies of lobe formation in mesophyll and epidermal cells of various plant species support a model in which lobe formation results from a nonuniform pattern of cellulose deposition directed by cortical microtubules [8–13]. Shortly before the initiation of lobes in both mesophyll and epidermal cells of various species, cortical microtubules become rearranged into bands thought to direct localized cellulose deposition, producing periodic cellulosic wall thickenings. The thinner regions of the wall between these thickenings are thought to extend more readily as cells expand under the force of turgor pressure, therefore bulging out to form lobes. Consistent with findings for other species, we found that the initiation of lobes in wild-type maize leaf epidermal cells is associated with a reorganization of cortical microtubules into transverse bands that are most conspicuous at the cell margins and become increasingly distinct as the lobes elongate (Figure 3A). Surprisingly, in spite of the absence of lobes, cortical microtubules are similarly organized into transverse bands in expanding *brk1* cells, although these bands are less distinct than in wild-type (Figure 3B).

Since differences in microtubule organization do not readily explain the complete lack of epidermal cell lobing in *brk1* mutants, we considered the role of F-actin in this process. In expanding wheat mesophyll cells, cortical F-actin was found to be arranged in bands coinciding with microtubule bands [14]. Treatment of these cells with low concentrations of cytochalasin D caused dispersion of microtubule bands, and the cells expanded without forming lobes, suggesting an important role for F-actin in promoting the formation of microtubule bands

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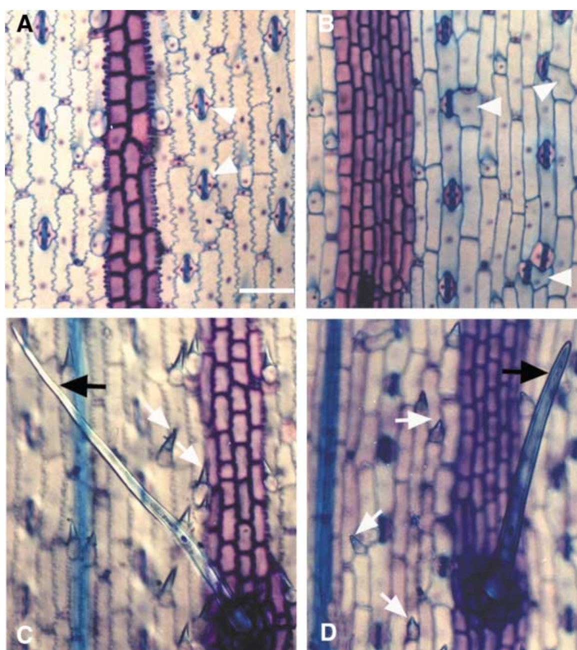

Figure 1. Epidermal Phenotypes of Wild-Type and *brk1* Leaf Blades

(A) wild-type epidermis showing normal stomata (white arrowheads) and epidermal pavement cells with interlocking marginal lobes.
(B) *brk1* epidermis with abnormal subsidiary cells (white arrowheads) and epidermal pavement cells lacking marginal lobes.
(C) wild-type epidermis with normal, sharply pointed prickle hairs (white arrows) and long, pointed macrohairs (black arrow).
(D) *brk1* epidermis with shortened, blunt prickle hairs (white arrows) and macrohairs (black arrow). Scale bar, 100 μm.

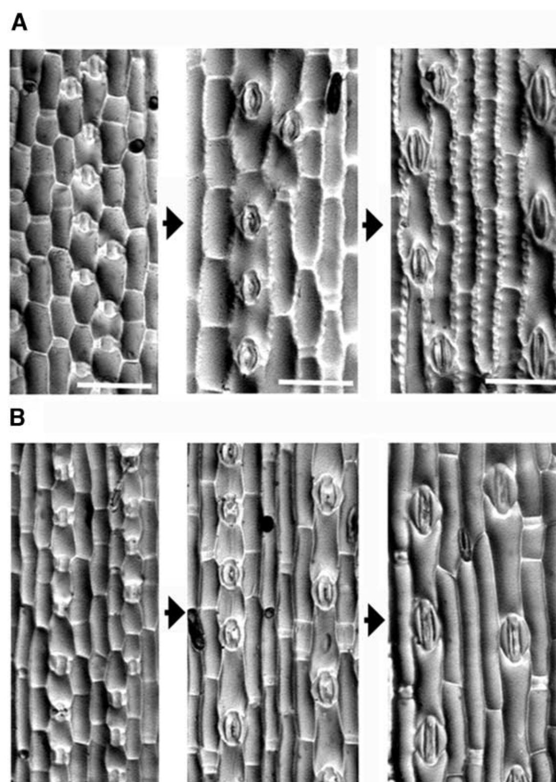

Figure 2. Cell Expansion in Wild-Type and *brk1* Mutant Leaf Blade Epidermal Cells

(A) wild-type; (B) *brk1*. The stages shown, from left to right, are found at ∼3 cm, 5 cm, and 7 cm, respectively, from the bases of 20–30 cm long leaves. Scale bar, 100 μm.

[15]. In contrast, a recent study of expanding *Arabidopsis* cotyledon epidermal cells showed that F-actin tended to be excluded from areas of the cell cortex where microtubule bands were observed [13]. Thus, there appears to be variability in the organization of F-actin in different lobe-forming cell types. In expanding wild-type maize leaf epidermal cells, cortical F-actin is enriched in distinct "patches" at sites of lobe emergence, which persist at lobe tips as the lobes elongate (Figures 3C and 3E). In expanding *brk1* epidermal cells, such F-actin patches are never observed (Figures 3D and 3F). These observations suggest that in addition to the banding of cortical microtubules, the formation of cortical F-actin patches along the cell margins is also critical for lobe formation in maize leaf epidermal cells. The absence of these F-actin patches could be responsible for the failure of lobes to form in *brk1* epidermal cells. Furthermore, the absence of this feature of F-actin organization in expanding wheat mesophyll cells may explain why *brk1* does not affect lobe formation in mesophyll cells. Moreover, in view of earlier results indicating an important role for F-actin in the organization of cortical microtubule bands in expanding wheat mesophyll cells [15], effects of *brk1* on cortical F-actin could account for the less distinct banding of microtubules in mutant cells. However, the fact that cortical microtubule bands are still present in expanding *brk1* epidermal cells in the absence of cortical F-actin patches suggests that these patches have other functions in lobe formation besides promoting the formation of microtubule bands.

The local enrichment of cortical F-actin observed in lobe tips is reminiscent of that seen at or near the growth site in tip-growing cells [3, 6, 16]. The significance of this feature of F-actin organization in tip-growing cells is not known, but various possibilities have been proposed [16], which might also explain the significance of F-actin enrichments at lobe tips. One possibility is that F-actin at lobe tips guides vesicle delivery to and/or promotes vesicle fusion with the plasma membrane. By analogy to migrating animal cells in which local F-actin polymerization propels the leading edge forward, another possibility is that F-actin polymerization at the lobe tip may drive its elongation by producing a protrusive force. Further work will be required to determine whether lobe formation shows other characteristics of tip growth. However, it is clear that *Brk1* is not required universally for tip growth. No obvious abnormalities are observed in *brk1* root hairs (data not shown). Moreover, mutant alleles are readily transmitted through the haploid male gametophyte, indicating that mutant pollen tubes can grow relatively normally.

Our observations concerning the effects of *brk1* on lobe formation, combined with results of an earlier analysis of the effects of *brk1* on stomatal development, suggest a unifying explanation for the role of *Brk1* in epidermal cell morphogenesis. Figure 4 illustrates the sequence of events leading to formation of wild-type and *brk1* stomata. Following an asymmetric division giving rise

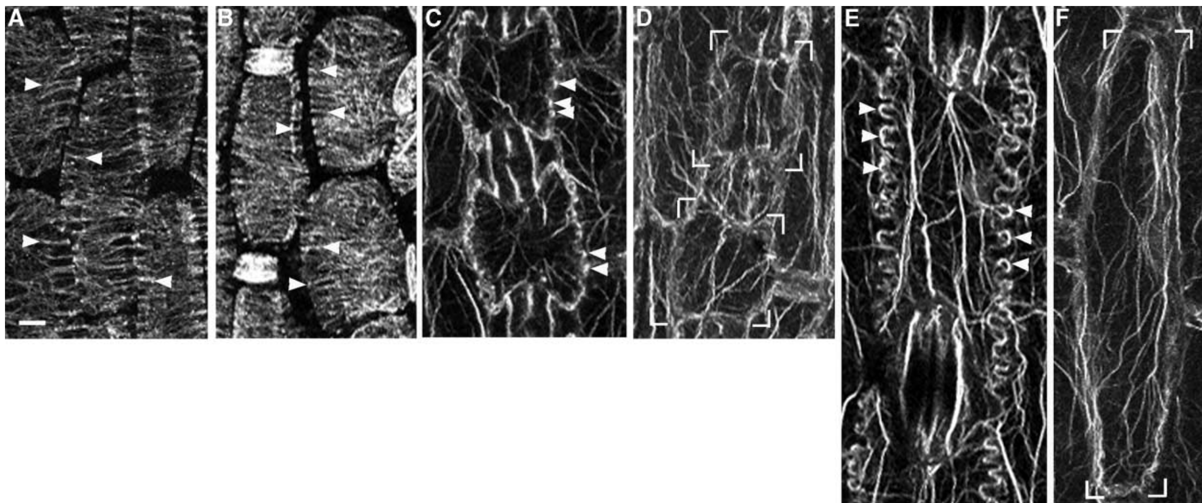

Figure 3. Analysis of Cytoskeletal Organization in Wild-Type and *brk1* Epidermal Cells

Microtubule organization in immature leaf tissue from wild-type (A) and *brk1* (B). White arrowheads indicate some areas where microtubules are banded. Actin organization at early and late stages of lobe formation in wild-type (C and E) and *brk1* (D and F) epidermal cells. White arrowheads in (C) and (E) point to some of the cortical F-actin enrichments associated with lobe formation. White brackets in (D) and (F) mark cell corners. Scale bar, 6 μm.

to a small guard mother cell (GMC), its nearest lateral neighbors (subsidiary mother cells, or SMCs) become polarized with respect to the GMC. The SMC's polarity is indicated by the position of its nucleus and the presence of an F-actin patch at the cortical site flanking the GMC. Subsequently, each SMC divides asymmetrically to form a small subsidiary cell adjacent to the GMC. Abnormal stomata in *brk1* mutants arise from SMCs that failed to form an actin patch or localize the nucleus asymmetrically [17]. Thus, whereas *brk1* epidermal pavement cells fail to establish polar growth sites, *brk1* SMCs often fail to become polarized prior to division.

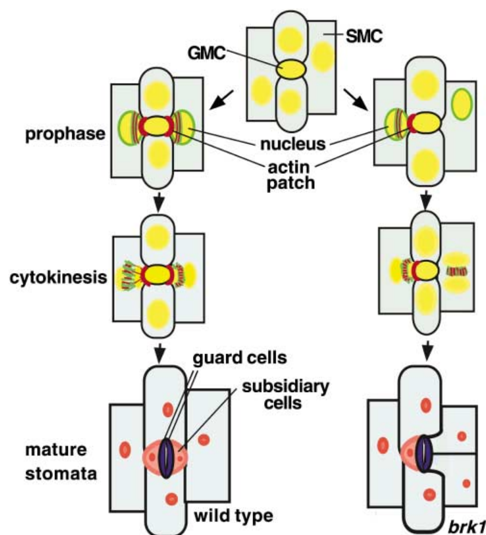

Figure 4. Schematic Summary of Defects in *brk1* Stomatal Development Reported in Reference 17

In developing stomata, nuclei are yellow, microtubules are green, and F-actin is red (see Results for additional explanation).

Both of these polarization events are characterized by local enrichments of cortical F-actin, which fail to form in *brk1* mutants. We have not investigated what cytoskeletal alterations may be associated with formation of the shorter, blunter hairs of *brk1* mutants compared to wild-type. However, recent work in *Arabidopsis* has demonstrated that initiation of trichomes and trichome branches depends primarily on microtubules, but elongation of trichome branches requires F-actin [18, 19]. Thus, all aspects of the *brk1* mutant phenotype we have observed could be due to a loss of actin-dependent polarization events in maize leaf epidermal cells.

Brick1 Encodes a Small, Novel Protein Highly Conserved in Plants and Animals

In order to gain more insight into the function of *Brk1*, a *Mutator* transposon tagged allele, designated *brk1-mum1*, was isolated to facilitate cloning of this gene (see the Supplementary Material available with this article online). A *Mu1*-containing XhoI fragment that cosegregated with the *brk1-mum1* mutant phenotype was cloned by screening a plasmid library constructed from DNA of *brk1-mum1* homozygotes with *Mu1* as a probe. A fragment of predicted exon sequence flanking the *Mu1* element was used as a probe to screen a leaf cDNA library. Three independent cDNA clones were isolated and sequenced. The 252 bp long coding region in each of these three cDNAs is identical and is composed of two exons (Figure 5A). Comparison with the size of the corresponding mRNA on Northern blots confirms that these cDNAs contain the entire coding region. Sequencing of the *brk1-1* allele revealed a single base pair change near the end of the first exon, which changes a glutamine codon to a stop codon. This confirms that the *Brk1* gene was cloned.

The pattern of *Brk1* gene expression was analyzed by Northern blot analysis (Figure 5B). *Brk1* RNA was

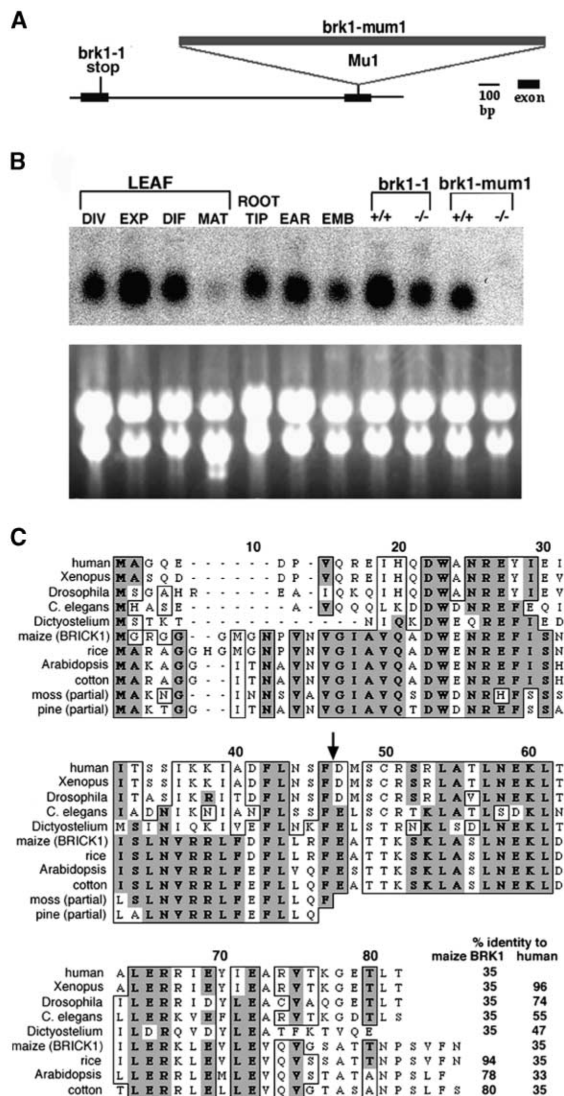

Figure 5. *Brk1* Encodes a Small, Novel Protein that Is Highly Conserved in Plants and Animals

(A) Schematic representation of the *Brk1* gene. The transcribed region of the gene is shown; filled boxes represent exons. The locations of the *Mu1* insertion in *brk1-mum1* and the premature stop codon in *brk1-1* are shown.

(B) Northern blot analysis of *Brk1* gene expression. Each lane was loaded with ∼15 μg of total RNA, and the blot was probed with a full-length *Brk1* cDNA. In leaves: dividing tissue (0–2 cm from base), DIV; expanding tissue (3–5 cm from base), EXP; differentiating tissue (6–8 cm from base), DIF; and mature tissue, MAT. EAR, ear primordia; EMB, embryo. RNA samples in the last four lanes were from the expanding leaf region of plants with the indicated genotypes.

(C) Ethidium bromide staining of ribosomal RNA in the gel transferred to the blot shown in (B), demonstrating approximately equal loading.

(D) Alignment of BRK1 amino acid sequence with related sequences in other eukaryotes (assembled with the Clustal W alignment tool in MacVector version 6.5.3). Residues shaded dark gray are identical (light gray similar) in >50% of proteins shown. Arrow shows location of a single intron found in each of the genomic sequences. GenBank accession numbers are as follows. Human, AF281279 (cDNA) and AC023236 (genomic); *Xenopus laevis*, BG515680 (cDNA); *Drosophila melanogaster*, AE003462 (genomic); *Caenorhabditis elegans*, CEY57G11C (genomic) and AU202036 (cDNA); *Dictyostelium discoidium*, AU074535 (cDNA); maize, AY093614 (cDNA), *Arabidopsis thaliana*, AC006340 (genomic) and AF370530 (cDNA); *Gossypium arboreum* (cotton), BF274937 (cDNA); *Pinus taeda* (pine), AW290353 (cDNA); *Physcomitrella patens* (moss), AW738961 (cDNA).

detected in all tissues analyzed: leaves, embryos, ear primordia, and roots. Notably, when leaf tissue was separated into dividing, expanding, differentiating, and mature regions, RNA levels were highest in expanding leaf tissue (the stage when cell lobing occurs) and were extremely low in mature leaf tissue. RNA levels were also analyzed in *brk1-mum1* and *brk1-1* homozygous mutants compared to their wild-type siblings. No mRNA was detected in *brk1-mum1* mutants, while slightly reduced RNA levels were detected in *brk1-1* homozygotes, consistent with the nature of these mutations.

The *Brk1* gene encodes a very small (∼8 kDa), novel protein with no recognizable functional motifs or targeting sequences. However, database searches show this protein to be highly conserved throughout the plant kingdom (Figure 5C). BRK1 is 96% identical to the rice ortholog, 78%–80% identical to dicot orthologs, and almost as highly conserved in the region of overlap with partial sequences from pine and moss. This family of plant proteins identifies a corresponding family that is also highly conserved throughout the animal kingdom. BRK1-related proteins from *Xenopus*, *Drosophila*, *C. elegans*, and *Dictyostelium* are 96%, 74%, 55%, and 47% identical to the human BRK1-like protein, respectively. Comparison of plant and animal proteins shows a high degree of sequence conservation in the center and divergence at the carboxy and amino termini, with 35% overall identity between BRK1 and any one of the animal proteins. Proteins in this previously unrecognized family are present in all eukaryotic genomes searched except those of fungi, including the fully sequenced genome of *S. cerevisiae*.

The conservation of both sequence and size of plant and animal proteins suggests that they function similarly in a process that was already occurring in the common ancestor of plants and animals. This process may have been dispensed with during the evolution of fungi or may involve a BRK1 ortholog too divergent at the sequence level to be recognizable. Further work will be needed to determine whether BRK1-like proteins also function to promote the polarization of animal cells. Our observations on the *brk1* phenotype in maize suggest that local F-actin polymerization is a critical feature of the cell polarization events in which this protein functions. Thus, one possibility for the biochemical function of BRK1 and related proteins in other organisms is to participate in the regulation of actin dynamics, a possibility that will be investigated in future studies.

Supplementary Material

Supplementary Material including Experimental Procedures is available at http://images.cellpress.com/supmat/supmatin.htm.

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References

1. Giddings, T.H., Jr., and Staehelin, L.A. (1991). Microtubule-mediated control of microfibril deposition: A re-examination of the hypothesis. In The Cytoskeletal Basis of Plant Growth and Form, C.W. Lloyd, ed. (London: Academic Press), pp. 85–99.
2. Cyr, R.J. (1994). Microtubules in plant morphogenesis: Role of the cortical array. Annu. Rev. Cell Biol. *10*, 153–180.
3. Hepler, P.K., Vidali, L., and Cheung, A.Y. (2001). Polarized cell growth in higher plants. Annu. Rev. Cell Dev. Biol. *17*, 159–187.
4. Dong, C.-H., Xia, G.-X., Hong, Y., Ramachandran, S., Kost, B., and Chua, N.-H. (2001). ADF proteins are involved in the control of flowering and regulate F-actin organization, cell expansion, and organ growth in *Arabidopsis*. Plant Cell *13*, 1333–1346.
5. Vidali, L., McKenna, S.T., and Hepler, P.K. (2001). Actin polymerization is essential for pollen tube growth. Mol. Biol. Cell *12*, 2534–2545.
6. Fu, Y., Wu, G., and Yang, Z. (2001). Rop GTPase-dependent dynamics of tip-localized F-actin controls tip growth in pollen tubes. J. Cell Biol. *152*, 1019–1032.
7. Baluska, F., Jasik, J., Edelmann, H.G., Salajova, T., and Volkmann, D. (2001). Latrunculin B-induced plant dwarfism: Plant cell elongation is F-actin-dependent. Dev. Biol. *231*, 113–124.
8. Jung, G., and Wernicke, W. (1990). Cell shaping and microtubules in developing mesophyll of wheat. Protoplasma *153*, 141–148.
9. Wernicke, W., Gunther, P., and Jung, G. (1993). Microtubules and cell shaping in the mesophyll of *Nigella damascena* L. Protoplasma *173*, 8–12.
10. Apostolakos, P., Galatis, B., and Panteris, E. (1991). Microtubules in cell morphogenesis and intercellular space formation in *Zea mays* leaf mesophyll and *Pilea cadierei*. J. Plant Physiol. *137*, 591–601.
11. Panteris, E., Apostolakos, P., and Galatis, B. (1993). Microtubules and morphogenesis in ordinary epidermal cells of *Vigna sinensis* leaves. Protoplasma *174*, 91–100.
12. Panteris, E., Apostolakis, P., and Galatis, B. (1994). Sinuous ordinary epidermal cells behind several patterns of waviness, a common morphogenetic mechanism. New Phytology *127*, 771–780.
13. Qiu, J.-L., Jilk, R., Marks, M.D., and Szymanski, D.B. (2002). The *Arabidopsis SPIKE1* gene is required for normal cell shape control and tissue development. Plant Cell *14*, 101–118.
14. Jung, G., and Wernicke, W. (1991). Patterns of actin filaments during cell shaping in developing mesophyll of wheat (*Triticum aestivum* L.) Eur. J. Cell Biol. *56*, 139–146.
15. Wernicke, W., and Jung, G. (1992). Role of cytoskeleton in cell shaping of developing mesophyll of wheat. Eur. J. Cell Biol. *57*, 88–94.
16. Geitman, A., and Emons, A.M.C. (2000). The cytoskeleton in plant and fungal cell tip growth. J. Microsc. *198*, 218–245.
17. Gallagher, K., and Smith, L.G. (2000). Roles for polarity and nuclear determinants in specifying daughter cell fates after an asymmetric division in the maize leaf. Curr. Biol. *10*, 1229–1232.
18. Mathur, J., Spielhofer, P., Kost, B., and Chua, N.-H. (1999). The actin cytoskeleton is required to elaborate and maintain spatial patterning during trichome cell morphogenesis in *Arabidopsis*. Development *126*, 5559–5568.
19. Szymanski, D.B., Marks, M.D., and Wick, S.M. (1999). Organized F-actin is essential for normal trichome morphogenesis in *Arabidopsis*. Plant Cell *11*, 2331–2348.

Accession Numbers

The maize Brk1 cDNA sequence has been deposited in GenBank with accession number AY093614.