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The *tangled-1* mutation alters cell division orientations throughout maize leaf development without altering leaf shape

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SUMMARY

It is often assumed that in plants, where the relative positions of cells are fixed by cell walls, division orientations are critical for the generation of organ shapes. However, an alternative perspective is that the generation of shape may be controlled at a regional level independently from the initial orientations of new cell walls. In support of this latter view, we describe here a recessive mutation of maize, *tangled-1* (*tan-1*), that causes cells to divide in abnormal orientations throughout leaf development without altering overall leaf shape. In normal plants, leaf cells divide either transversely or longitudinally relative to the mother cell axis; transverse divisions are associated with leaf elongation and longitudinal divisions with leaf widening. In *tan-1* mutant leaves, cells in all tissue layers at a wide range of developmental stages divide transversely at normal frequencies, but longitudinal divisions are largely substituted by a variety of aberrantly oriented divisions in which the new cell wall is crooked or curved. Mutant leaves grow more slowly than normal, but their overall shapes are normal at all stages of their growth. These observations demonstrate that the generation of maize leaf shape does not depend on the precise spatial control of cell division, and support the general view that mechanisms independent from the control of cell division orientations are involved in the generation of shape during plant development.

Key words: cytokinesis, leaf development, maize, *tangled-1*

INTRODUCTION

The question of how plants acquire their shapes has intrigued plant developmental biologists for many years. Plant cells are constrained by their walls such that the relative positions of cells within tissues are established by the orientation of new walls at cytokinesis. As a consequence, cell division orientations are traditionally assumed to be crucial for the generation of organ shapes (eg. Ashby, 1948; Stebbins, 1967; Brown, 1976; Furuya, 1984; Fosket, 1990). According to this traditional view, in which cells are the building blocks of morphogenesis, the elaboration of shape depends upon a series of properly oriented cell divisions, together with appropriate patterns of cell expansion. A variety of observations have been interpreted as support for this view. Many descriptive studies have emphasized the predictable relationships that often exist between patterns of cell division and morphogenesis. For example, cells divide in a highly predictable pattern during the initial stages of embryogenesis in *Capsella* and *Arabidopsis*, (Johanson, 1950; Wardlaw, 1955; Mansfield and Briarty, 1991). Similarly, cell divisions in the root tip of the aquatic fern, *Azolla*, are invariant in their orientations (Gunning et al., 1978). Additional evidence shows that changes in cell division orientations frequently predict subsequent changes in the direction of overall growth. For example, the predominant orientation of cell division in the prospective leaf-forming region of the shoot apical meristem shifts from anticlinal to periclinal just before the emergence of a new leaf primordium (Esau, 1977; Lyndon, 1983). Similarly, changes in the orientation of cell division in the root pericycle forecast the emergence of lateral root primordia (Esau, 1977; Casero et al., 1993). These observations demonstrate that there is often a close correlation between patterns of cell division and morphogenesis, but do not firmly establish a causal role for cell division orientations in the generation of form.

An alternative perspective is that shape is acquired by means of the spatial control of growth at a regional level in a manner that is largely independent from the division orientations of individual cells. In support of this view, Kaplan and Hagemann (1991) demonstrate that many species of algae acquire shapes that resemble those of vascular plants, despite distinctly different patterns of cell divisions. Indeed, even within an individual species, overall shape can be generated uniformly despite variability in the underlying pattern of cell division. For example, in contrast to the predictable patterns in *Capsella* and *Arabidopsis*, cell divisions are randomly oriented during cotton and maize embryogenesis (Pollock and Jensen, 1964; Randolph, 1936). Analyses of genetic chimeras also show that

minor variations in the pattern of cell division are a normal feature of leaf development in several plant species (eg., Stewart et al., 1974; Stewart and Dermen, 1975; Poethig, 1987). Compelling additional evidence for a degree of independence between cell division and morphogenesis comes from a series of studies in which cell division was arrested experimentally in developing shoots and roots of wheat (Haber, 1962; Haber and Foard, 1963; Foard et al., 1965; Foard, 1971). These studies demonstrate that leaf and lateral root primordia can initiate, and existing leaves can undergo properly oriented (albeit limited) growth, in the complete absence of cell division (Haber, 1962; Haber and Foard, 1963; Foard et al., 1965; Foard, 1971). If shape is generated by means of the spatial control of growth at a regional level, cells may normally be stimulated to divide as a response to increases in tissue and thus cell volume. Cell division orientations could thus be a consequence rather than a cause of the direction of organ growth, particularly because simple geometric rules can often predict the orientation of cell division from the shape of the mother cell (reviewed by Lloyd, 1991; Barlow, 1991; Cooke and Lu, 1992). That is, cell division within plant tissues could for the most part be simply a reduction of cell volume according to processes that favor the production of cells of characteristic shapes.

Analysis of mutants that alter either morphogenesis or cell division pattern provides a useful opportunity to investigate the causal relationships between these two processes. In many morphological mutants, altered cell division patterns accompany, and are often assumed to be the cause of, abnormal morphogenesis. For example, mutations altering both the length/width ratio (*lam*) and the thickness (*fat*) of tobacco leaves are attributed to changes in cell division pattern during early leaf development (McHale, 1993). Abnormalities in cell division patterns during embryogenesis of *Arabidopsis* are interpreted as the cause of morphological defects in the mutants *gnom*, *monopteros* and *fass* (Mayer et al., 1993; Berleth and Jurgens, 1993; Torres-Ruiz and Jurgens, 1994). However, according to the alternative perspective outlined above, alterations in cell division pattern could also be interpreted as a secondary consequence of abnormal morphogenesis in these mutants rather than a cause, or as an independent effect of the mutation. When both processes are affected, the cause and effect relationships between shape generation and cell division pattern remain unclear. Here we describe a mutant of maize, *tangled-1* (*tan-1*), in which cells in all tissue layers of the leaf divide in abnormal orientations at all stages of leaf development, but the overall shape of the leaf is nevertheless acquired normally. The characteristics of this mutant demonstrate that cell division orientations are not critical for the elaboration of normal maize leaf shape, and support the general view that the generation of plant shape can be controlled independently from cell division pattern.

MATERIALS AND METHODS

Plant material

The *tan-1* mutation arose in a *Mutator* stock derived from one originally provided by Don Robertson (Iowa State University). Mutants were crossed to several different inbred lines and the subsequent progeny self-pollinated to confirm that the mutant phenotype segregates consistently as a single Mendelian recessive trait. To produce the material for the studies described here, two cycles of crosses to the inbred line A188 followed by self-pollination of the resulting progeny were performed to generate material in which the *tan-1* mutation was segregating in a genetic background that was approximately 3/4 A188 overall. In every experiment described, comparisons were made between *tan-1* mutant and normal segregants from the same family grown under identical conditions in the greenhouse. All data presented are for leaves 8, 9 and 10 (counting the first seedling leaf as leaf 1), which in the genetic background used for these studies were adult leaves of very similar size and growth characteristics.

Histology

Blade tissue from mature leaves 8, 9 and 10 was cleared by treating sequentially with 95% ethanol (1-3 days), 1 N NaOH (16 hours or overnight), 25% bleach (1 hour), and finally saturated chloral hydrate (16 hours or longer). Following water rinses, cleared tissues were stained overnight in an aqueous 0.01% toluidine blue solution. Cleared, stained leaves were mounted in water between two microscope slides with the adaxial surface facing up, and photographed under bright-field conditions on a Zeiss Axiophot microscope with Kodak Ektar 25 film.

For sectioning, leaf tissue was fixed in 4% formaldehyde/100 mM NaH_2PO_4 pH 7, dehydrated through an ethanol series, embedded in JB-4 resin (Polysciences, Warrington, PA), and sectioned at 2 μm on a Microm microtome using a tungsten carbide knife. Sections were attached to poly-L-lysine coated slides. Cross sections through the blades of mature leaves 8, 9 and 10 were stained for several minutes in an aqueous 0.05% toluidine blue solution, mounted in water and photographed as described in the previous paragraph. Paradermal sections of leaf 8, 9 and 10 primordia 0.6-2.0 cm long were stained with a calcofluor white preparation (undiluted Fungifluor, Polysciences, Warrington, PA), rinsed briefly in distilled water, then stained for several minutes in an aqueous 1 μg/ml solution of 4,6-diamidino-2-phenylindole (DAPI, Sigma, St. Louis, MO). Following 15-60 minutes of destaining in distilled water, sections were mounted in water, examined on a Zeiss Axiophot microscope under UV epi-illumination with a standard DAPI filter set, and photographed with Kodak T-Max 400 film.

Division orientations in paradermal sections were determined by examining cells at two stages: the first were cells in late telophase, when the cell plate was visible but still incomplete. The second were cells that had recently completed cytokinesis as indicated by the presence of a closely spaced pair of condensed nuclei on opposite sides of a cell wall. The orientations of these forming or recently formed walls were scored relative to the long axis of the mother cell. New walls that formed an angle of less than 30° with a line perpendicular to the mother cell's long axis were scored as transverse. New walls that joined the two ends of the mother cell were scored as longitudinal. Other divisions were assigned to the classes indicated in each figure.

Scanning electron microscopy (SEM)

Replicas of the leaf surface for SEM analysis were prepared essentially as described previously (Williams and Sylvester, 1994). Briefly, impressions of the adaxial leaf surface from leaves 8, 9 and 10 at the stages indicated were made in polysiloxane dental impression medium (Exaflex Injection Type, Patterson Dental Supply, Sunnyvale, CA), and fitted into molds that were used to cast replicas of the leaf surface from Spurr's epoxy resin (Polysciences, Warrington, PA). Epoxy replicas were coated with gold-palladium in a sputter coater, and analyzed on a Hitachi scanning electron microscope at an accelerating voltage of 20 kV. Micrographs were taken of the leaf impressions using 4X5 Polaroid film. The Polaroid positive was analyzed following methods described by Sylvester et al. (1990). In brief, cross-walls were determined to be recently formed based on shallow wall depth relative to the adjoining two perpendicular mother cell walls.

The orientations of shallow cross walls were scored in relation to the long axis of the mother cell as described in the previous section, with one exception: longitudinal divisions in which the new wall joined the two ends of the mother cell in a crooked or curved path were only observed in mutants, so they were scored as aberrant and placed in their own category (see Fig. 4C).

Morphometric analysis

Growth rates of mutant and normal sibling leaves were determined by measuring the lengths of leaves 8, 9, and 10 daily, beginning on the first day the leaf tip became visible (at approximately 30% of its final length) and for each of ten consecutive days thereafter, during which time they reached nearly full length.

The shapes of mutant and normal sibling leaves were compared by recording and measuring their 2-dimensional outlines at successive stages of growth. Leaves 8, 9 and 10 were removed at stages ranging from 1 cm to full size, unrolled, flattened with the aid of double stick tape, and traced. Length and maximum width measurements were taken directly from these tracings.

RESULTS

The *tangled -1* mutant phenotype

The *tangled-1* (*tan-1*) mutation arose in a maize stock with active *Mutator* transposons; it segregates as a single gene recessive trait after outcrossing into various inbred lines for several generations. Mutant plants are similar in their overall appearance to normal siblings except that they are generally shorter in stature with smaller, more erect leaves than normal (Fig. 1B vs. A). In addition, mutant leaves have a distinctly roughened, crepe-papery texture compared to the smooth surface of a normal leaf (Fig. 1D vs. C).

The surface view shown in Fig. 2A illustrates that the normal adult maize leaf epidermis consists of a highly regular array of linear cell files. The majority of epidermal cells are rectangular, non-specialized cells whose long axes are aligned with each other. A variety of specialized epidermal cells, including stomata, hairs, and bulliform cells, are also formed in regular, linear patterns. The vascular network in the internal tissues of the leaf is visible as an evenly spaced, parallel array of longitudinal veins interconnected by short, transverse veins (Fig. 2A). Cross sections of normal leaf tissue illustrate that each vein consists of vascular elements arranged in a characteristic pattern, surrounded by a single-celled ring of bundle sheath cells. Longitudinal veins are typically separated from each other by two mesophyll cells; intermediate longitudinal veins are typically separated from the adaxial and abaxial epidermis by one mesophyll cell (Fig. 2C).

All aspects of this normal cell pattern are altered in *tan-1* mutant leaves. As illustrated in Fig. 2B, mutant epidermal cells are variable in shape, and are not well aligned, giving the epidermis a disorganized appearance. The regular distribution of stomata, hairs and bulliform cells is correspondingly disrupted. Veins appear to be tangled in a network rather than uniformly patterned as in normal leaves. Mutant leaf cross sections also illustrate the irregular placement of veins, relative to the epidermis as well as to each other (Fig. 2D). As in the epidermis, all differentiated cell types of the internal tissue layers are present, but their shapes and their arrangement within the tissue are irregular compared to normal. Other plant organs, including roots, stems and floral organs are similarly affected (data not shown), demonstrating that the *tan*$^{+}$ gene functions throughout the plant rather than in a single tissue or organ type.

Cell division orientations during maize leaf development

The origin of the altered cell pattern observed in *tan-1* mutant leaves was investigated by examining the position of new cell walls at cytokinesis. Previous analyses of cell division during maize leaf development (Sharman, 1942; Poethig, 1984; Sylvester et al., 1990) have established the general scheme represented in Fig. 3, which illustrates the approximate distribution and orientation of cell division in the leaf epidermis as the primordium grows to its final size and shape. The majority of cell divisions are transverse, with the new wall perpendicular to the long axis of the leaf, or longitudinal, with the new wall parallel to the long axis of the leaf. Transverse divisions are associated with leaf elongation, and longitudinal divisions with leaf widening (Sylvester et al., 1990). From inception to the 1 cm stage represented in Fig. 3B, cells divide in both transverse

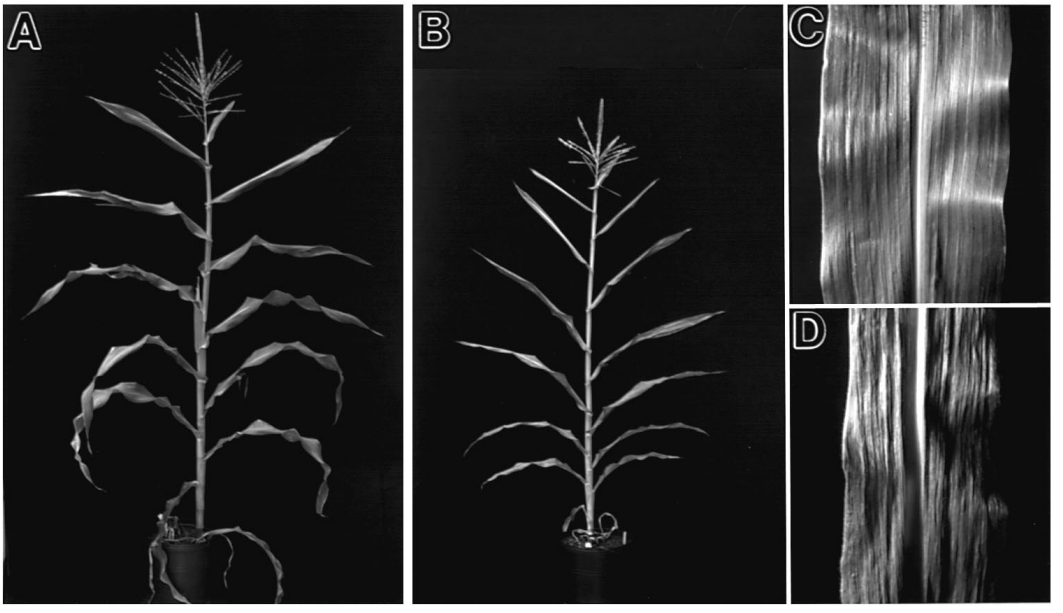

Fig. 1. Macroscopic features of the *tan-1* mutant phenotype. Photographs (same scale) illustrating the overall appearance of a fully grown normal plant (A) and its *tan-1* mutant sibling (B). At closer range, the rougher texture of an adult *tan-1* mutant leaf (D) compared to its normal sibling leaf (C) can be observed.

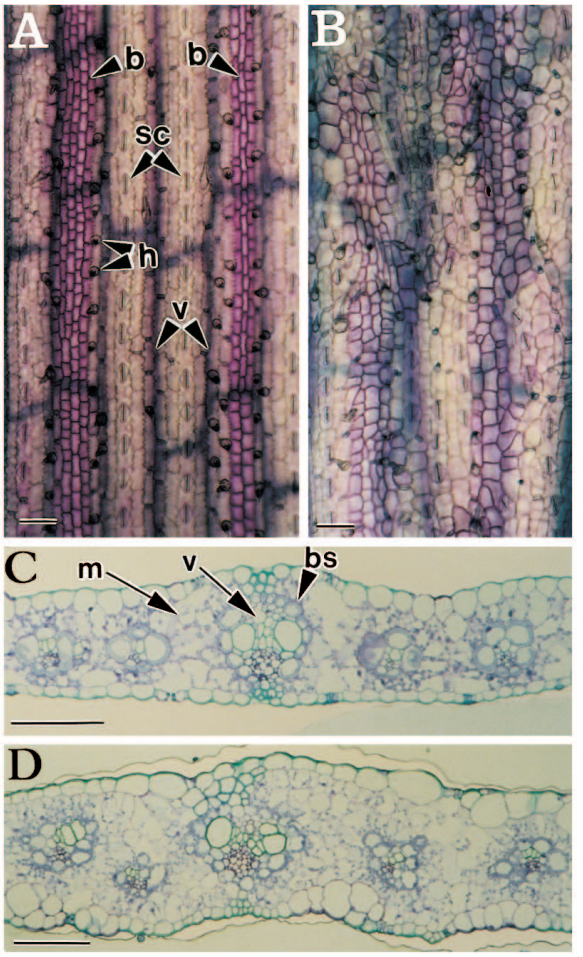

Fig. 2. Histological features of normal and *tan-1* sibling adult leaves. Cleared leaves, adaxial surface up (A,B), and transverse sections (C,D) of of mature normal (A,C) and *tan-1* (B,D) leaves, showing alterations in the shape and arrangement of cells in all tissue layers. In A, arrowheads indicate the location within the epidermis of bulliform cells (b), stomatal complexes (sc), and hairs (h); v indicates a vein in the middle leaf layer. In C, one vein (v) and its associated bundle sheath (bs) are indicated. Scale bars, 100 μm.

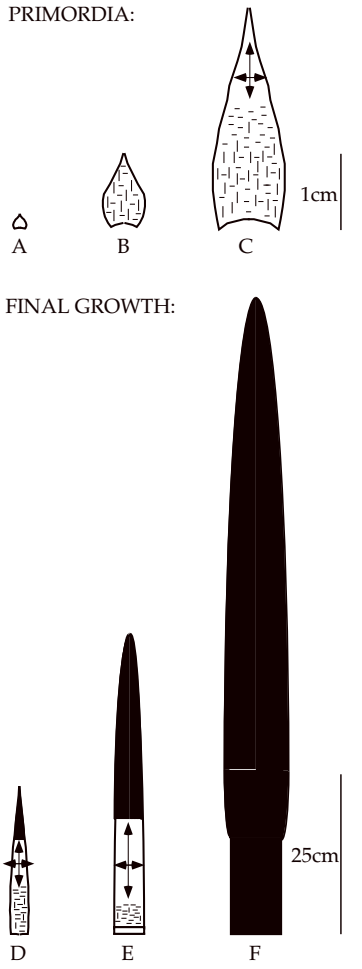

Fig. 3. Schematic representation of the approximate distribution and orientation of cell divisions in the adaxial epidermis of normal maize leaves as they grow from 1 cm primordia (B) to final size (F). Data based on descriptions of Sharman (1942), Sylvester et al. (1990), and unpublished observations (A. W. S. and L. G. S.). In B-E, short black lines represent cell divisions in transverse (horizontal lines) and longitudinal (vertical lines) orientations. Following the cessation of cell division, cells continue to expand postmitotically, as indicated by double-headed arrows in C-E. In mature regions of the leaf, represented by solid black, cells have stopped expanding and are terminally differentiated. Leaf development proceeds basipetally such that cells continue to divide in the basal region of the leaf after cells in the distal region have already ceased dividing, expanding and undergone maturation.

and longitudinal orientations throughout the entire primordium (indicated by the short, black lines, Fig. 3B). During this interval, the primordium increases more than ten-fold in length. Subsequently, divisions are gradually restricted to the base of the growing leaf (and to the transverse orientation) while cells in the distal region continue to expand postmitotically (as indicated by the arrows in Fig. 3C, D and E). Leaf maturation (when cell expansion has ceased and cells terminally differentiate, indicated by solid black in Fig. 3D-F) begins at the leaf tip and spreads gradually toward the leaf base. Thus, cells at the leaf base are the last to stop dividing, expanding and to undergo maturation.

The effects of *tangled-1* on cell division orientations during leaf development

The positions of new walls in recently divided epidermal cells were examined in normal and *tan-1* mutant leaf primordia approximately 1 cm long, a stage at which cells are still dividing and expanding throughout the entire primordium (represented in Fig. 3B). In scanning electron micrographs of replicas of the epidermis, newly formed walls appear as shallow indentations in the leaf surface (Fig. 4A,B, arrows). The epidermal cells of normal leaf primordia are rectangular with their long axes aligned parallel to the long axis of the leaf. Quantitative analysis of recent cross-walls shows that 51% of the new walls in the epidermis of normal 1 cm leaf primordia are transverse (perpendicular to the long axis of the mother cell), and the remaining 49% are longitudinal (parallel to the long axis of the mother cell; Fig. 4A, arrowheads; 4C). Most mutant epidermal cells at this stage are also elongated, but are more variable in shape, and their long axes are frequently skewed in relation to

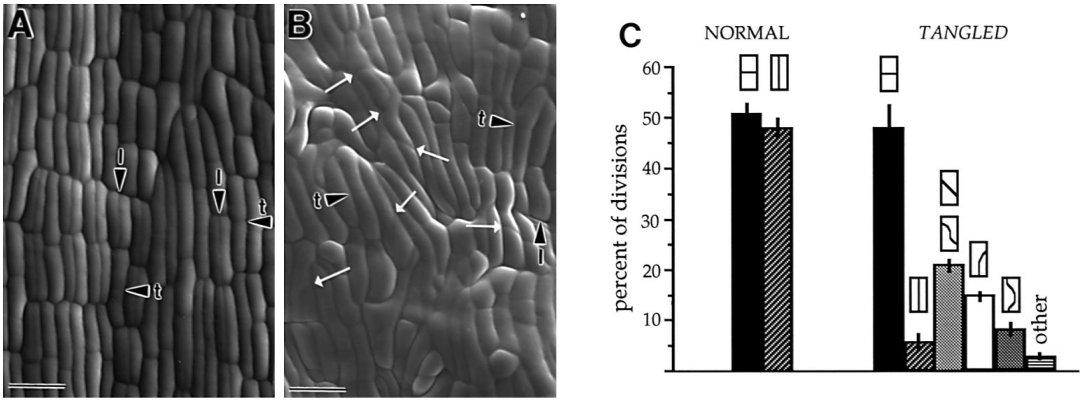

Fig. 4. Analysis of recent cell divisions in the adaxial epidermis of normal and *tan-1* sibling leaf primordia 0.5-1.5 cm long (the stage represented in Fig. 3B). Micrographs are oriented with the long axis of the leaf aligned vertically. In both A (normal) and B (*tan-1*), arrowheads point to shallow indentations indicative of recent transverse (t) and longitudinal (l) divisions. In B, white arrows point to aberrant recent divisions observed in *tan-1* primordia. Scale bars, 30 μm. (C) Quantitative analysis of recent cell divisions visualized in scanning electron micrographs. For this analysis, a minimum of 300 shallow walls were scored in relation to the long axis of the mother cell in micrographs representing several dispersed sites on the leaf surface for each of three normal and three mutant primordia. Divisions were assigned to the classes indicated; error bars indicate standard errors.

the leaf axis (Fig. 4B). Similar to normal primordia, 48% of new walls in the epidermis of *tan-1* primordia are transverse relative to the mother cell's long axis (Fig. 4B, arrowheads; 4C). However, longitudinal divisions are reduced from 49% in normal to 5% in mutant primordia, and are replaced by a variety of aberrant divisions producing crooked or curved new walls that are rarely observed (<1%) in the epidermis of normal primordia. These aberrant divisions were assigned to the categories illustrated in Fig. 4C; several examples are indicated by white arrows in Fig. 4B.

Cell division orientations in internal tissue layers were examined in paradermal sections of normal and mutant leaf primordia at the same 1 cm stage. In normal primordia, 61% of the observed divisions are transverse, and 33% are longitudinal (Fig. 5A). Examples of these two types of divisions in a normal leaf primordium are shown in Fig. 5B and C (arrowheads). In addition, a few percent of divisions observed in normal primordia are of other types (Fig. 5A). Similar to normal leaves, 58% of divisions in the internal tissues of mutant leaves at the 1 cm stage are transverse. However, the proportion of longitudinal divisions is reduced from 33% in normal to 8% in mutant primordia, and a corresponding increase is observed in the proportions of aberrant divisions of the same classes described earlier for the mutant epidermis (Fig. 5A). Examples of transverse and longitudinal divisions within a *tan-1* leaf primordium are shown in Fig. 5D and E, respectively (arrowheads), and examples of the aberrant divisions observed most frequently in *tan-1* leaf primordia are shown in Fig. 5F-H (white arrows).

This analysis was extended to examine cell division orientations at other stages of leaf development; dividing cells at all stages examined were found to be similarly affected by the *tan-1* mutation. For example, cell division orientations were examined in leaves that are 50% of their final length, a stage

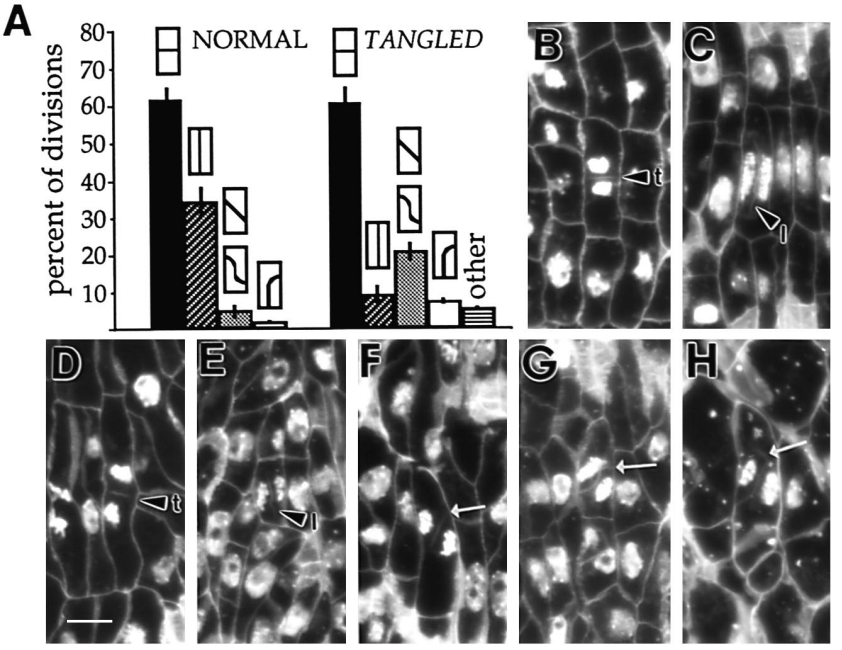

Fig. 5. Analysis of cell division in the internal tissue layers of normal and *tan-1* sibling leaf primordia 0.6-2 cm long (the stage represented in Fig. 3B). Paradermal sections (cut in the plane of the leaf surface) were stained with calcofluor white and DAPI to fluorescently label cell walls and nuclei, respectively. (A) Division orientation relative to the mother cell's long axis was scored for a minimum of 200 non-epidermal cells in telophase (cell plate present but incomplete) or immediately post-telophase (cell plate complete but nuclei still concensed and paired on opposite sides of the cell plate) for each of three normal and three mutant leaf primordia; divisions were assigned to the classes indicated. Error bars represent standard errors. In B-H, micrographs are oriented with the long axis of the dividing cell aligned vertically. Examples of normal transverse (t) and longitudinal (l) divisions in normal primordia (B,C) and in *tan-1* primordia (D,E), are indicated by arrowheads. In F-H, examples of aberrant divisions commonly observed in *tan-1* leaf primordia are indicated by white arrows. Scale bar, 10 μm.

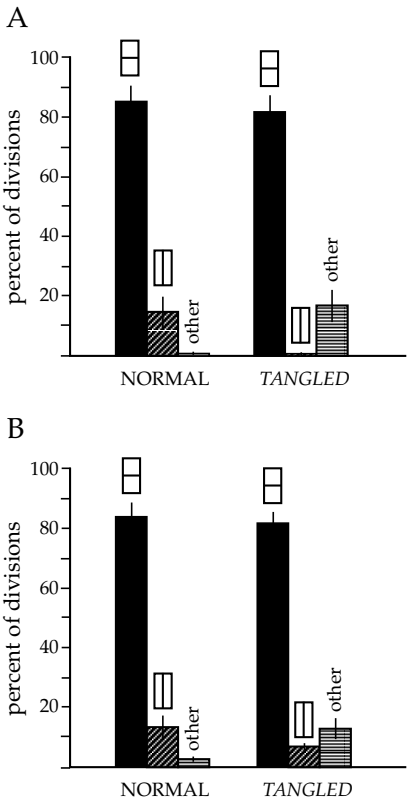

Fig. 6. Quantitative analysis of cell division orientations within 1 cm of the base of the leaf blade of normal and *tan-1* sibling adult leaves at approximately 50% of their final length (the stage represented in Fig. 3E). To produce the data presented in A, at least 200 adaxial epidermal divisions were scored in scanning electron micrographs of 3 normal and 3 mutant leaves as described in the legend to Fig. 4C. For B, at least 200 divisions in the internal tissue layers of 3 normal and 3 mutant leaves were analyzed in fluorescently labelled paradermal sections as described in the legend to Fig. 5A, except that only 136 divisions were scored for one of the 3 normal leaves. Error bars represent standard errors.

when cell division is restricted to the basal portion of the leaf (represented in Fig. 3E). Analysis of scanning electron micrographs shows that in normal leaves, 86% of recent epidermal cell divisions are transverse and the remaining 14% are longitudinal (Fig. 6A). In *tan-1* mutant leaves at this stage, a similar proportion (82%) of recent epidermal divisions are transverse, whereas longitudinal divisions are reduced to less than 1% and the remaining 18% of recent divisions are aberrant (Fig. 6A; in this case, all aberrant divisions were grouped together into a single category, 'other', to simplify the figure). The corresponding analysis of division orientations in internal tissues at the same stage shows a similar trend: in wild-type leaves, 84% of divisions are transverse, 13.5% are longitudinal, and 2.5% are classified as 'other.' In mutant leaves, 82% of divisions are transverse, whereas longitudinal divisions are reduced to 6%, and the proportion of 'other' divisions is increased to 12% (Fig. 6B). A similar spectrum of aberrantly oriented divisions was observed in mutant primordia at the 1-2 mm stage depicted in Fig. 3A (data not shown).

In summary, analysis of cell division orientations at a wide range of developmental stages shows that in all tissue layers

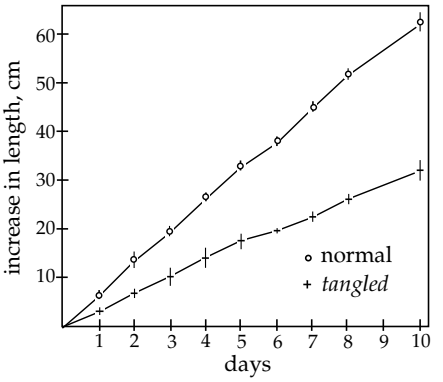

Fig. 7. Comparison of normal and *tan-1* mutant leaf growth rates. Daily measurements of leaf length were made for 8 normal and 8 mutant adult leaves beginning on the day their tips were first visible (approximately 30% of final length) and for 10 consecutive days thereafter, by which time they had generally reached near final size. The length on the first day was used as a reference point, and increases from that initial length were plotted as a function of the number of days since the initial measurement was taken. Error bars represent standard errors.

of *tan-1* mutant leaves, transverse cell divisions occur at normal frequencies, but longitudinal divisions are largely substituted by a variety of aberrantly oriented divisions.

The effects of *tangled-1* on maize leaf morphogenesis

Although *tan-1* mutant leaves are generally smaller than normal, the mutation appears to have little effect on the overall shape of mature leaves. The effects of the mutation on leaf morphogenesis were evaluated in two ways. First, to determine whether the smaller size of mutant leaves reflects a slower growth rate, daily increases in leaf length were recorded for mutant and normal sibling leaves as they grew from 30% to near 100% of their final length. The results, presented in Fig. 7, illustrate that the rate at which mutant leaves elongate during this interval is approximately half of normal. Second, to examine the effects of the mutation on the elaboration of leaf shape, the two-dimensional outlines of *tan-1* and normal leaves ranging from 0.5 cm to final length were traced and directly compared (Fig. 8A). The tracings show that normal maize leaves grow allometrically from the 1 cm stage to their final size, with a constant three-fold increase in length for every two-fold increase in width. The leaf therefore becomes progressively longer and thinner, changing from the spade-like shape at the 1 cm stage to the elongate strap-like shape at maturity. Normal leaves reach an average final length of 100 cm in the A188 genetic background, whereas mutant sibling leaves are approximately half as long at maturity. These observations, combined with our prior results that mutant leaves grow from 30% to near final size at half the normal rate (Fig. 7), are consistent with the view that the duration of growth and the maturation rate of mutant leaves is normal. When tracings of mutant leaves are compared with those of normal leaves of the same length, their shapes are always very similar (Fig. 8A). To quantify the generation of leaf shape in normal and mutant plants, measurements of leaf length and maximum width were used to generate standard allometric growth curves, in which the log of leaf length is plotted against the log of leaf width over the entire

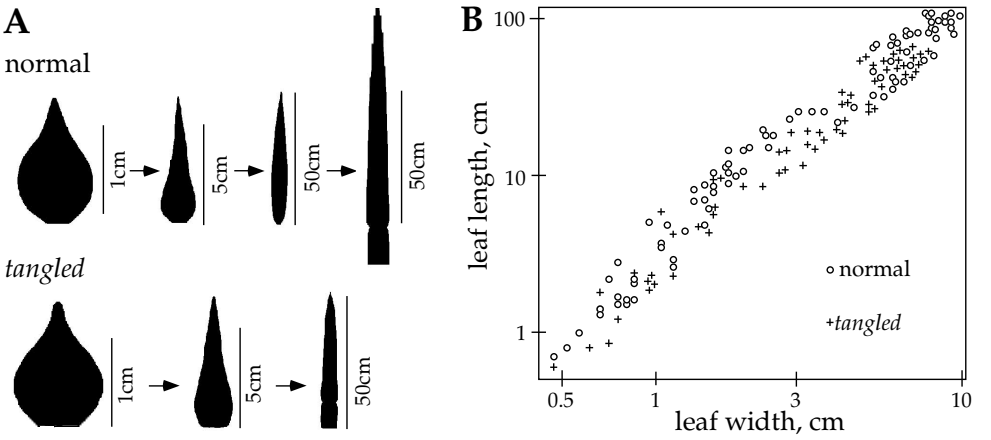

Fig. 8. Analysis of adult leaf morphogenesis in normal and *tan-1* mutant plants. Leaf tracings were made of a wide range of stages from <1 cm to final size. In A, representative tracings of selected stages of normal and mutant leaf growth are shown (note changes of scale). For B, length and maximum width measurements were taken from leaf tracings and plotted on a double logarithmic plot to generate the allometric growth curves shown.

growth period (Fig. 8B). Due to the constant rate of increase in length relative to width, the resulting growth curve is linear. The growth curves for normal and mutant leaves are superimposable, illustrating again that *tan-1* leaf morphogenesis proceeds normally, albeit at a slower than normal rate.

DISCUSSION

In this study, we have analyzed the effect of a recessive mutation, *tan-1*, on cell division orientations during maize leaf development, and the consequences of this mutation for the generation of leaf shape. Previous studies showed that during the formation of a normal maize leaf, transverse divisions correlate with leaf elongation and longitudinal divisions with leaf widening (Poethig, 1984; Sylvester et al., 1990; Poethig and Szymkowiak, 1995). Here we show that this correlation is uncoupled by the *tan-1* mutation. In mutant leaves, cells in all leaf layers at a wide range of developmental stages examined divide transversely at normal frequencies, but the majority of longitudinal divisions are substituted by aberrantly oriented divisions in which the new cell wall is crooked or curved. Mutant leaves grow more slowly and their final size is smaller than normal, but their shapes at all stages of leaf growth are similar to those of normal leaves of the same length. Thus, despite the near complete absence of normal, longitudinal cell divisions, the rate of growth in length relative to width is correctly regulated in mutant leaves, and their shapes are elaborated normally. These observations demonstrate that the generation of leaf shape in maize does not strictly depend on the control of cell division orientations, and imply that the generation of shape and the control of cell division orientations in this organ are separable processes that can be independently controlled, the tan^+ gene being required only for the latter process.

Earlier studies of leaf morphogenesis in another grass, wheat, have already demonstrated that new leaf primordia can initiate, and existing leaves can undergo properly oriented growth, in the absence of cell division (Haber, 1962; Haber and Foard, 1963; Foard, 1971). These pioneering studies, which were the first to demonstrate experimentally a separation between cell division and plant morphogenesis, are limited by the fact that relatively little growth occurred after the cessation of cell division, presumably because sustained growth is dependent upon the formation of new cells. Our observations confirm and extend the results of these earlier studies by demonstrating properly oriented leaf growth during a prolonged period during which the leaf increased over 100-fold in length in the continuous presence of abnormally oriented cell divisions. As elegantly discussed previously (Haber, 1962; Haber and Foard, 1963; Foard, 1971), these observations support the general view that shape during plant development is acquired independently from the pattern of cell division.

These results raise many questions concerning how the spatial control of plant growth is achieved and also what mechanisms are responsible for orienting plant cell division. Our observations imply that morphogenetic information in the maize leaf is distributed such that individual cells, regardless of their division orientations and shapes, expand in the proper orientation relative to the leaf as a whole. However, we can only speculate as to the nature of the information that dictates shape. One possibility is founded in biochemistry: properly oriented growth depends on the spatial distribution of diffusible substances such as hormones or other growth stimulating activities yet to be characterized. Another possibility is founded in biophysics: the spatial control of growth depends upon the distribution of physical stresses, which create the appropriate strain within a growing tissue to favor growth in one orientation over another (eg., Green, 1987; Kutschera, 1989). As elegantly discussed by Green (1994), the biochemical and biophysical explanations are not necessarily mutually exclusive. In our view, a synergistic, and perhaps obligate, relationship could well exist between the chemical signals/responses for morphogenesis and the physical signals/responses imposed by the nature of the developing organ. How shape is generated during development remains as an intriguing problem that is not well understood in any organism. In animals, where relative cell positions within tissues are not constrained as they are in plants, morphogenesis is thought to involve both cell migration and the regional coordination of cell shape changes. Morphological mutants in *Drosophila* are providing relevant information concerning the mechanisms underlying both processes (eg., Parks and Wieshaus, 1991; Young et al., 1993; Costa et al., 1994). Similarly, morphological mutants that alter organ shapes might

provide further insight into mechanisms of plant morphogenesis (eg., Sinha et al., 1993; Benfey and Schiefelbein, 1994).

The cytoskeleton is definitively implicated as a key player in the process by which plant cells position their division planes (Wick, 1991). However, little is known about how cells choose a division plane that is appropriate for their developmental context, and how that choice is translated into the formation of properly positioned cytoskeletal arrays involved in cell division. The cell division defects seen in *tan-1* mutants could well have a cytoskeletal basis. Specifically, the selective effect of this mutation on longitudinal cell divisions implies that the TAN^+ gene product could be directly involved in the process by which a longitudinal division plane is chosen and/or executed in cells of elongated shape. Thus, ongoing analysis of the cytoskeletal rearrangements associated with cell division in *tan-1* mutants combined with molecular analysis of the TAN^+ gene product could provide new information about mechanisms governing the orientation of plant cell division.

Recently, other mutants have also been described in which cells divide abnormally without affecting various aspects of plant development that are often assumed to depend on the precise control of cell division. *Arabidopsis fass* mutations (Torres-Ruiz and Jurgens, 1994) and the phenotypically similar mutations *ton-1* and *ton-2* (Traas et al., 1995), cause abnormally oriented cell divisions during embryogenesis and abnormal morphogenesis, producing a longitudinally compressed, radially enlarged seedling. However, the basic body plan in both apical-basal and radial dimensions is normal. In addition, dominant negative cdc2 kinase mutations have been shown to partially suppress cell division during tobacco development without affecting organ shapes; plants having these mutations consist of fewer cells that have expanded more, as if to compensate for their smaller numbers (Hemerly et al., 1995). Together with the results reported here, these observations show that in the appropriate mutants, the control of cell division can be uncoupled from both morphogenesis (*tan-1* mutants and dominant negative cdc2 kinase mutants) and pattern formation (*fass*, *ton-1*, *ton-2*).

Nevertheless, it is clear that plants do utilize precise mechanisms for controlling the timing of cell division and the position of division planes. What is the significance of cell division orientations during plant development, if not for the definition of overall shape, or for the establishment of the body plan? Perhaps the clearest answer to this question is that, due to the lack of relative cell movement in plant tissues, cell division pattern during plant development has a significant impact on the cellular order of plant tissues, and thus potentially on many aspects of tissue function. In this respect, cell division pattern may be particularly important late in development, when the last divisions occur that establish the final relative positions of cells within the plant. For example, among the last cell divisions to occur in the leaf epidermis are those leading to the formation of stomata. A coordinated sequence of divisions results in the formation of multicellular stomatal complexes in which the configuration of cells is important for their function in gas exchange with the environment (Raschke, 1975). Thus, it may be that the developmental process on which cell division orientations have the greatest impact is histogenesis, or the formation of organized, functional tissues.

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Note added in proof

Recent results show that the *tan-1* mutation maps to the same chromosomal region as the *pigmy plant 1* (*py1*) mutation, and also fails to complement *py1*. Thus, these two mutations are allelic. The *py1* mutation, which arose spontaneously, was reported by A. D. Suttle in an unpublished Ph.D. thesis: 'The Genetic Interrelations of Different Types of Dwarf Corn' (Cornell University, 1924).