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Clonal Sectors Reveal That a Specific Meristematic *narrow sheath*

Michael J. Scanlon and Michael Freeling[1]
Department of Plant Biology, University of California, Berkeley, California 94720

The narrow leaf and shortened stem phenotypes of the maize mutant narrow sheath (ns) are postulated to result from the lack of founder cell initialization in a region of the meristem that gives rise to leaf and stem margins. To test this model, a lineage map of the maize meristem is presented which compares the development of leaf margins in the narrow leaf mutant, narrow sheath (ns), and wild-type sibling plants. X-irradiation of mature seeds produced aneuploid albino sectors in wild-type and ns mutant plants. Of particular interest are sectors occurring in more than one leaf, which reflect a meristematic albino cell lineage. Analyses of these sectors indicated that: (1) a region of the ns meristem does not contribute to the founder cell population of the incipient leaf; (2) the margins of ns mutant leaves are derived from a lateral region of the meristem different from those in wild-type siblings; (3) founder cells in wild-type, juvenile-staged vegetative meristems encircle the meristem to a greater extent than do founder cells in adult-staged meristems; and (4) meristematic leaf founder cells may be subdivided into specific lateral domains, such that the position of a sector on the meristem correlates with a particular cell lineage. These data support our model for *ns* gene function in a specific domain of the meristem.

INTRODUCTION

Genetically mosaic sectors are useful tools to study organ initiation and growth (Stein and Steffenson, 1959; Satina and Blakeslee, 1941; Poethig, 1984). Clonal sector analyses are particularly applicable to plants, because their rigid cell walls preclude cellular migration and allow cell lineage relationships to be relatively easily deciphered. Several fate mapping studies have been performed on the maize plant, with analyses concentrated on leaves, vasculature, flowers, anthers, the embryo, and the shoot apical meristem (Steffenson, 1968; Poethig, 1984; Cerioli *et al.*, 1994; Poethig and Szymkowiak, 1995; Langdale *et al.*, 1989; Johri and Coe, 1983; Dawe and Freeling, 1992; Poethig *et al.*, 1986; McDaniel and Poethig, 1988). These studies have contributed much to our current understanding of maize shoot development.

The maize vegetative shoot is segmented into repeating structural units termed phytomers. In maize, the phytomer (Fig. 1) comprises the leaf, node, internode, and subtending bud with its prophyll (Galinat, 1959; Bossinger *et al.*, 1992). Maize segments exhibit distichous phyllotaxy, meaning that successive phytomers form approximately 180° apart from each other and in two ranks. Phytomer organogenesis initiates at specialized regions (herein referred to as ''flanks'') located on opposite sides of the shoot apical meristem. Previous clonal analyses (Coe and Neuffer, 1978; Johri and Coe, 1983; Poethig, 1984; Poethig *et al.*, 1986) have shown that although their fates are slightly variable, cells in the maize shoot apical meristem have predictable destinies. In this way, the flank of the shoot apical meristem that gave rise to the midrib region of one leaf will give rise to the margin region of the next leaf to proliferate from the apex (Fig. 2). Fate mapping has indicated that the maize leaf is derived from approximately 250 founder cells which are recruited from the shoot apical meristem in an overlapping ring (Poethig, 1984). This founder cell population is probably 2 to 3 cell tiers high, about 30 cells in circumference, and occupies at least two meristematic cell layers (Poethig and Szymkowiak, 1995). Different regions of the founder cell population are recruited at different times, beginning at the premidrib flank and proceeding toward the premarginal flank of the meristem (Sharman, 1942; Poethig and Szym-

[1] To whom correspondence should be addressed. Fax: (510) 642-4995.

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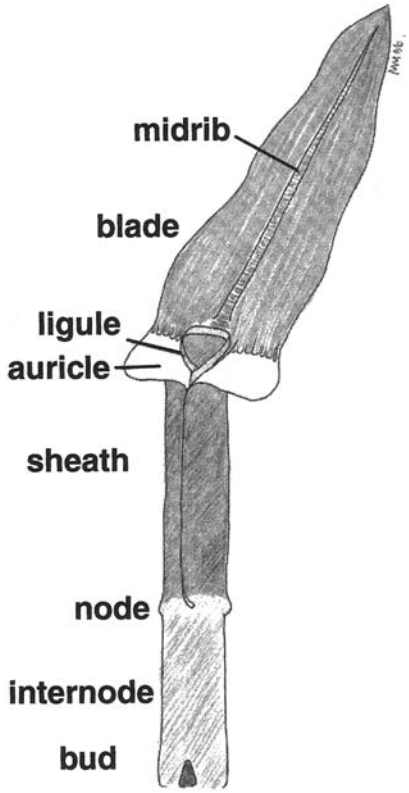


FIG. 1. Cartoon of the maize phytomer. The vegetative structural unit is comprised of the leaf, node, internode, and lateral bud. The main components of the leaf (sheath, ligule, auricle, midrib, and blade) are indicated.

kowiak, 1995). The accumulation patterns of the maize homeodomain protein KNOTTED1 (Smith *et al.*, 1992; Jackson *et al.*, 1994), a marker of leaf/nonleaf identity in the meristem, are consistent with the predictions of founder cell recruitment, number, and position obtained from previous studies.

During the primordial stage of growth, all founder cell derivatives divide approximately equally to effect an expansive, uniform growth phase (Poethig, 1984; Sylvester *et al.*, 1990). The full complement of leaf lateral veins are already present before the end of the primordial stage of phytomer development (Sharman, 1942). Leaf sectors frequently are bordered by lateral veins, which suggests that the vasculature may serve as boundaries to lateral compartments in the maize leaf (Cerioli *et al.*, 1994). During postprimordial development, the maize leaf differentiates basipetally (from the tip toward the base). Thus, leaf expansion first occurs in the upper leaf (blade), and later is localized to the lower leaf (sheath) (Poethig, 1984; Sylvester *et al.*, 1990; Poethig and Szymkowiak, 1995) and finally to the internode (Sharman, 1942; Poethig and Szymkowiak, 1995). Postprimordial growth is also characterized by expansive, cell divisions near the leaf margins (Steffenson, 1968; Poethig, 1984).

The narrow sheath (ns) mutant has been characterized previously as a duplicate factor trait that may represent the preprimordial deletion of a lateral domain of the phytomer (Scanlon *et al.*, 1996). Structures normally found at leaf margins are absent from ns mutant leaves, and the internodes are shortened on the margin side. The ns mutant phenotype is more severe in juvenile and early adult leaves, and less pronounced in leaves that are formed later on the plant. Although the size, shape, and histology of ns mutant meristems are indistinguishable from nonmutant siblings, the pattern of KNOX (KNOTTED-like homeodomain protein, a marker of leaf/nonleaf identity) accumulation in the margin flank of the founder cell ribbon is altered (Scanlon *et al.*, 1996). The ns mutant meristem fails to downregulate KNOX accumulation in the margin flank of the incipient

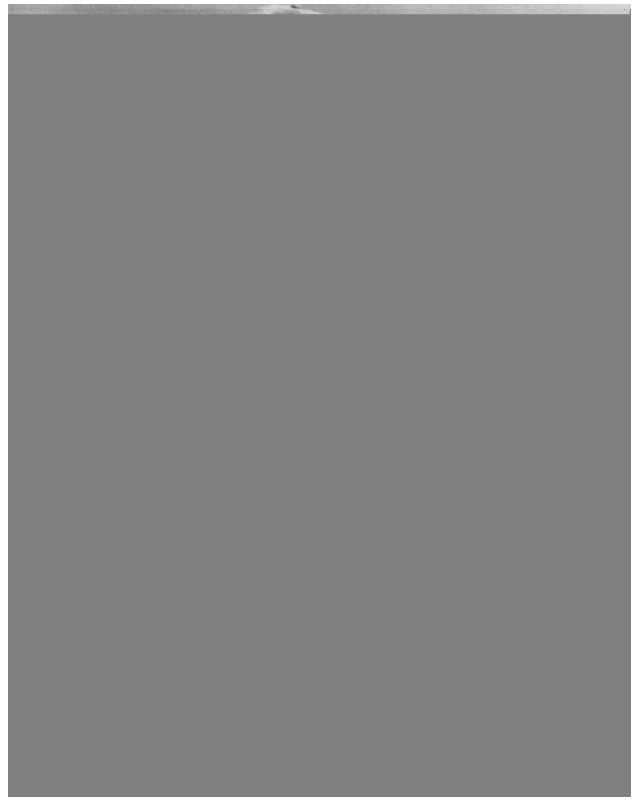


FIG. 2. Scanning electron micrograph of the maize shoot apex, illustrating the distichous phyllotaxy of grasses. Successive leaf primordia, designated as P1 (plastochron 1) and P2 (plastochron 2), are initiated from two specialized organogenic regions (termed flanks) on opposite sides of the shoot apical meristem (SAM). Therefore, an albino sector induced on one flank of the meristem (indicated by arrow) will have marked the midrib region of the P1 leaf primordium and the margin region of the P2 primordium. The scale bar represents 36 μm.

segment. We proposed a model whereby the *ns* genes function to initialize cells in the premargin region of the meristem to become leaf founder cells. According to this model, cells on the premargin flank of the meristem remain uninitialized in ns mutants, and are excluded from the founder cell population of the incipient phytomer. The result of this exclusion of margin founder cells is the deletion of margin domains from the leaf and internode.

In order to test this model further, we present a lineage analysis of the maize meristem, focusing on the early development of the margin domains of the phytomer. Albino sectors were generated on or near the flanks of the shoot apical meristems of narrow sheath and wild-type sibling embryos to compare cell lineage relationships in nonmutant versus mutant meristems. Because they were caused by a one-time X-ray treatment, the sectors are assumed to reflect individual, single-cell lineages created in the embryonic shoot. Two features of maize leaf development are especially useful in studies of meristematic cell lineage. These features are: (1) the distichous phyllotaxy of maize, wherein leaves are placed opposite and offset from each other (Fig. 2); and (2) the founder cell ribbon overlaps the meristem much like a key ring (Steffenson, 1968; Poethig, 1984). Therefore, one can estimate the positions of founder cell domains in the vegetative meristem by observing the positions of albino sectors located on two successive mature phytomers derived from a sectored meristem. We show that the edges of ns mutant leaves are not derived from the same lateral region of the meristem as in nonmutant sibling plants, and demonstrate that cells in the premargin flank of the ns mutant meristem do not contribute to the mutant leaf. Evidence for the subdivision of the founder cells into lateral domains is presented. Additional data suggest that as the meristem enlarges, the relative positions of the founder cell domains change from phytomer to phytomer. These data provide further evidence that the *ns* mutations condition a deletion of margin regions of the phytomer and support the model for *ns* gene function in a premargin, meristematic domain.

MATERIALS AND METHODS

Genetic Stocks and Clonal Analysis

The ns mutant phenotype is a duplicate factor trait dependent upon homozygosity for each of the two unlinked, recessive mutations *ns1* and *ns2* (Scanlon *et al.*, 1996). The plants used to construct genetic mosaics were generated by crossing ns mutant plants of the genotype *ns*1/*ns*1; *ns*2/*ns*2 onto plants heterozygous for either of the unlinked albino mutations *lw*1 or *lw*2. The F1 plants of the respective genotypes *ns*1/*Ns*1; *ns*2/*Ns*2; *Lw*1/*lw*1; or *ns*1/*Ns*1; *ns*2/*Ns*2; *Lw*2/*lw*2 were backcrossed to ns mutant plants. A total of 2400 seeds were generated. One half of the progeny of this cross were expected to be heterozygous for an albino mutation, and one quarter were expected to yield ns mutant plants. At the time of irradiation, embryos dissected from mature seed produced approximately 5 to 6 leaf primordia. The seeds were imbibed overnight and subjected to 1017 rad of X-irradiation over 3.2 min, through a 0.35-mm Cu filter with a Philips Model RT250 X-ray machine run at 225 kV. The irradiated seeds were planted in the Summer nursery in Santa Clara, California and plants were screened for the presence of meristematic (leaf to leaf), clonal sectors of the presumed genotype *lw*/−. Such meristematic, aneuploid sectors were identified by clones of albino tissue that were present on successive leaves as described previously (Steffenson, 1968). A total of 35 wild-type plants, and 18 ns mutants were found to contain meristematic sectors.

The sectored plants were harvested and scored in the following manner. The node number, the lateral vein number, length and width (to the nearest 0.5 mm) of each node, internode, leaf sheath, and blade containing a sector were recorded. Measurements were made at the midlength of the internode and sheath, and at the base of the leaf blade just above the auricle (Fig. 1). The width of the sector, and its distance from the margin and the midrib of the phytomer were measured differently in the node, internode, and sheath, than in the leaf blade. In the node/internode and sheath the distance to the sector was measured to the nearest 0.5 mm. In the leaf blade, however, the width of the sector and its distance to the midrib and margin were counted as the number of lateral vein distances spanned. The vasculature of the maize leaf includes the large central midvein and approximately 40–48 smaller lateral veins distributed about the midvein (Sharman, 1942). The lateral veins are themselves spanned by even smaller intermediate veins (see Fig. 3). A full complement of lateral veins form in maize leaves during the uniform growth period of primordial development (Sharman, 1942; Sylvester *et al.*, 1990). Afterward, the maize leaf blade undergoes nonuniform, postprimordial development, marked by expansive growth near the blade margin (Sharman, 1942; Poethig, 1984). Owing to this growth pattern, we judged that the number of lateral veins between a sector and the margin or midrib of the blade may more accurately reflect the actual position of a meristematic sector at the time of its induction.

In the graphical representations of the sectored phytomers, the width of the sector was plotted as the number of lateral veins the sector spanned. Likewise, the distance from the sector to the midrib and margin of the leaf blade was plotted as the number of lateral veins between the sector and those leaf structures. Measurements of the leaf sheath, node, and internode were plotted as the percentage of the total width in terms of lateral veins. For instance, a sector that is 4 mm wide and 1 cm from the margin of a leaf sheath that measures 5.0 cm from midrib to margin of a leaf and contains 20 lateral veins per one-half leaf is plotted as being 1.6 veins (4 mm wide/50 mm per half-sheath × 20 veins per leaf) wide and 4 veins (1 cm from the margin/5 cm per half-sheath × 20 veins per leaf sheath) from the sheath margin.

Dimensions of Plants

Plants from an inbred stock obtained from Pioneer Hi-Bred Intl. (Scanlon *et al.*, 1996) and segregating for ns mutant and nonmutant phenotypes were grown to maturity, and measurements of leaf width, leaf length, lateral vein number, internode length, and internode circumference were recorded for phytomers 10 and 14. The data were collected from five ns mutant and five nonmutant siblings and the average values for each category were calculated.

TABLE 1
Dimensions of Narrow Sheath Mutants

Character	ns Mutant		Nonmutant sibling	
	Phytomer 10	Phytomer 14	Phytomer 10	Phytomer 14
Lateral veins per $\frac{1}{2}$ leaf (*n*)	10	12	24	26
Leaf length (cm)	90	85	107	97
Sheath length (cm)	15.7	11.5	16	11.2
Sheath width at $\frac{1}{2}$ length (cm)	2.4	3.0	4.5	4.8
Width at ligule (cm)	1.4	1.8	6.6	8.0
Blade width at $\frac{1}{2}$ length (cm)	1.0	3.6	6.7	8.4
Blade width at widest (cm)	3.0	4.6	6.7	8.4
Internode length (cm)	6.8	12.0	14.5	17.5
Internode width (cm)	6.1	4.6	6.3	5.0

Microscopy

Light and fluorescence microscopy was performed on freehand, transverse sections of leaves and internodes as previously described (Becraft and Freeling, 1994). Scanning electron microscopy was performed on replicas of shoot apices dissected from 2-week-old seedlings as previously described (Sylvester *et al.,* 1990).

RESULTS

Dimensions of Narrow Sheath vs Nonmutant Sibling Leaves

The most striking phenotypes of ns mutant plants are narrow leaves and short, curved stems (Table 1, Fig. 3). Both phenotypes are attributed to the deletion of a margin domain of the phytomer (Scanlon *et al.,* 1996). The reduction in leaf width is more extreme in the sheath and lower blade than in the upper blade. Another feature of ns mutants is the alleviation of the leaf and stem phenotypes in upper nodes (compare phytomers 10 and 14 in Table 1). Despite the reduction in leaf width and the stem curvature seen in ns mutants, the leaf length and stem circumference are nearly identical to those in nonmutant siblings (Table 1). Also, the number of lateral veins (defined under Materials and Methods) in ns mutant leaves is reduced by more than 50% (Scanlon *et al.,* 1996).

Although the number of lateral veins in ns mutants is reduced by more than one half, the distances between successive lateral veins are similar to those seen in nonmutant siblings. Transverse sections of leaf 10 were made between the eighth and ninth lateral vein from the midrib of wild-type and ns mutant plants (Fig. 3C). There were 25 intermediate veins between lateral veins in the ns mutant leaf, and 24 intermediate veins between the lateral veins in the nonmutant sibling. Furthermore, Fig. 3C shows that the distances between intermediate veins are the same in ns mutant and nonmutant sibling leaves. Therefore, in the measurements of sectors presented below, the distance of a sector from the ns leaf midrib, as reported in terms of lateral vein number, is comparable to the distance from the midrib of a wild-type leaf sector spaced the same number of lateral veins from the midrib.

Meristematic Sector Types Observed in Nonmutant Control Plants

Of approximately 2400 X-rayed seeds, we recovered 35 nonmutant plants which also had meristematic sectors (identified as sectors which traversed from leaf to leaf, Steffenson, 1968; Poethig, 1984) of albino tissue. A feature common to all sectored plants was the tendency of meristematic sectors to meander about the midvein of the leaf. That is, the position of the sector with respect to the midrib showed varying degrees of alteration in successive leaves on the same side of the plant (Fig. 4F, 4D, and 6C). Previous studies have attributed this sector meandering to a shift in the midrib axis in some leaf primordia, such that new leaf primordia are not always initiated exactly 180° apart from each other (Poethig, 1984; Steffenson, 1968). Sectors in nonmutant plants fell into four major classes. These sector types were described as phytomer pairs; the symbols ⟨ ⟩ are used to designate a single phytomer, and a phytomer pair is designated as ⟨ ⟩⟨ ⟩. The sector classes are designated herein as: (1) ⟨on midrib⟩⟨sheath margins⟩; (2) ⟨near midrib⟩⟨both sheath margins and one blade margin⟩; (3) ⟨off midrib⟩⟨off margin⟩; and (4) saddle sectors (Poethig *et al.,* 1986). Descriptions of these sector types are outlined below.

(Wild-type sector class 1) ⟨on midrib⟩⟨sheath margins⟩ sectors. Albino sectors which fell on the midrib of one leaf and appeared near both margins of the sheath, but not the blade, of an adjacent leaf were observed on three separate plants in this study (Figs. 4A, 4B, and 5A–5C). The sheath margins sectors passed close to both the right and left edges of the sheath; usually the sector fell closer to one sheath

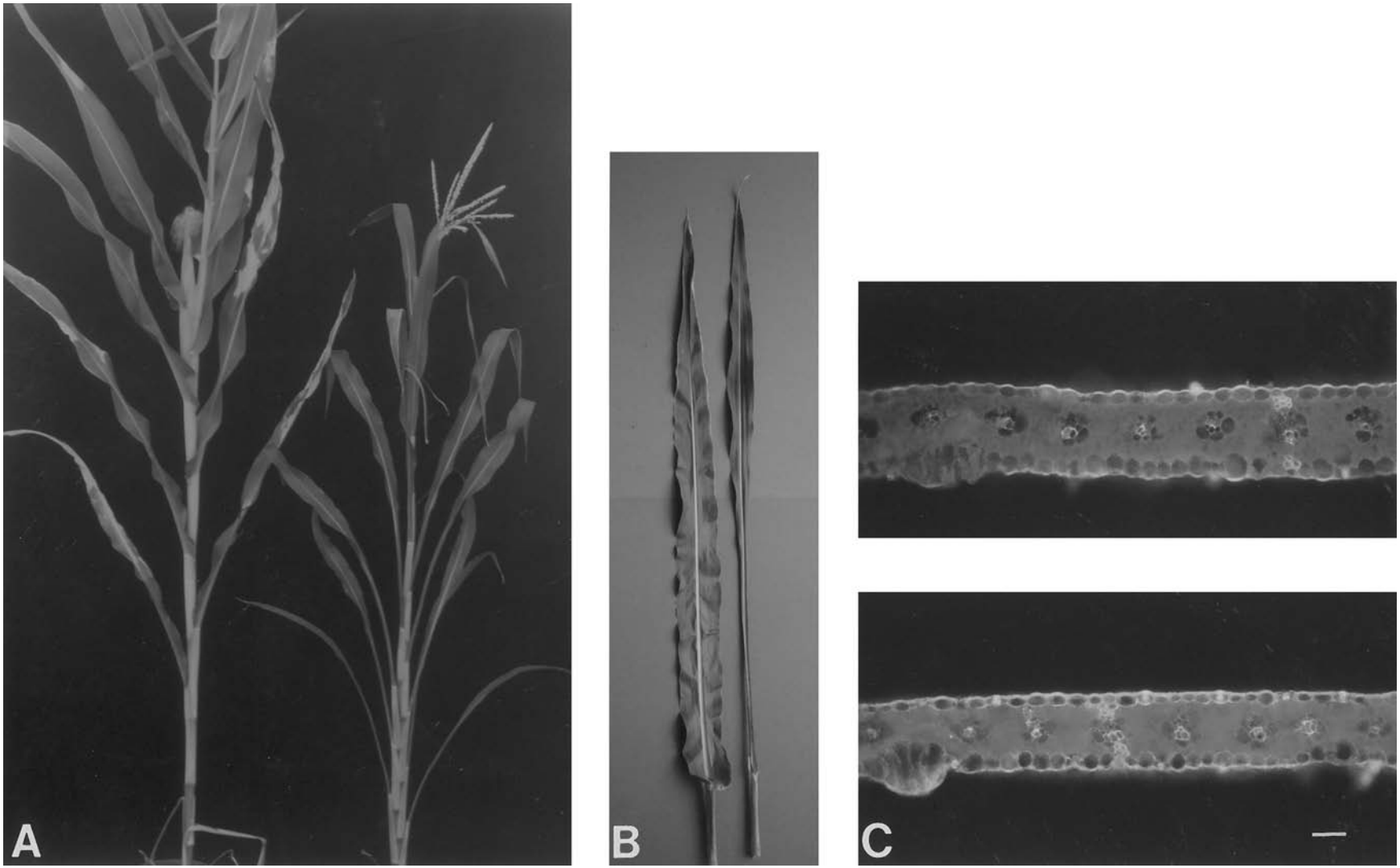


FIG. 3. Morphology of narrow sheath mutant plants. (A) Nonmutant (left) and narrow sheath (ns) (right) mutant plants. The ns mutants are shorter, primarily due to decreased internode length. (B) Adult leaves from nonmutant and ns mutant plants. The ns mutant leaf is much thinner in the sheath and lower blade, although the length is normal. (C) Fluorescence micrograph of transverse sections of ns mutant (top) and nonmutant sibling (bottom) leaf 10 showing intermediate veins between the eighth and ninth lateral veins from the midrib. The intervein spacing is not disturbed in the mutant leaf. The scale bar represents 160 μm.

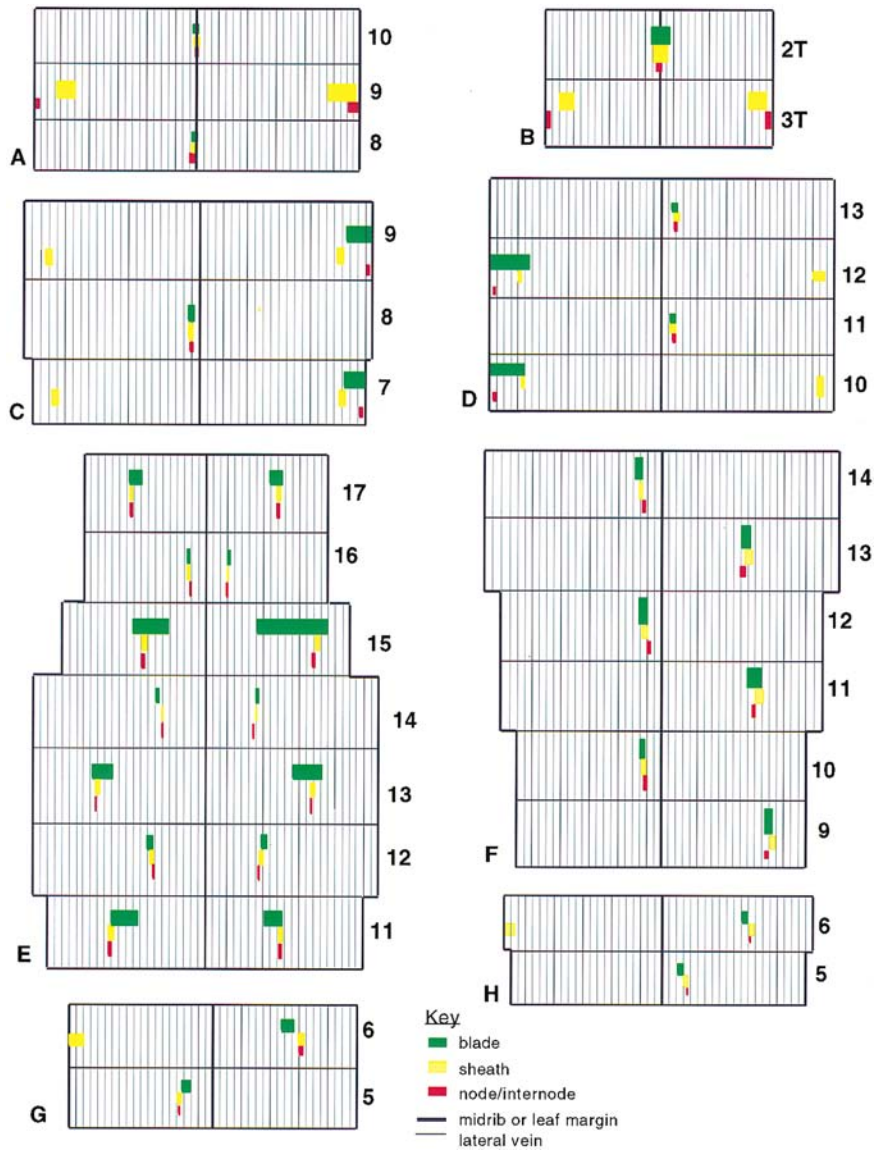
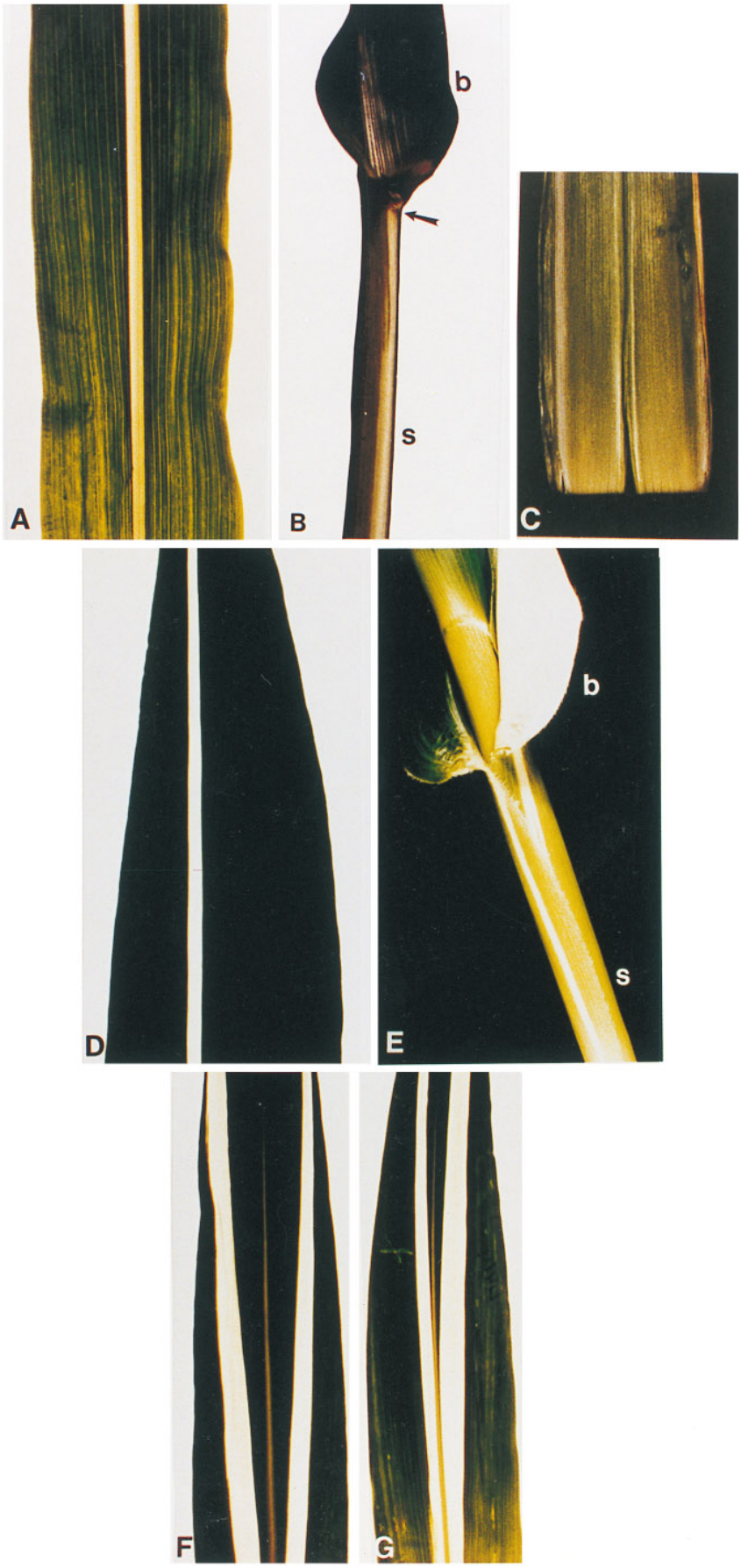


FIG. 4. Diagrams of representative sectors observed in nonmutant plants. Each grid represents successive phytomers containing a sector. The numbers to the right of each grid indicate the segment (phytomer) number. The distances of each sector from the midrib and margin were determined as described under Materials and Methods. The center midrib and both outer margins of each phytomer are designated as thick vertical lines, whereas the lateral veins are shown as thinner lines. (A) An ⟨on midrib⟩⟨sheath margins⟩ sector that passed through the midrib of the blade, sheath, and node/internode of phytomers 8 and 10, and passed through the node/internode, and sheath of leaf 9. Note that in leaf 9 the sector passed through both margins of the sheath at a position interior to the edge, but missed the leaf blade. (B) Another ⟨on midrib⟩⟨sheath margins⟩ sector that appeared in a nonmutant tiller (lateral branch) and showed the same characteristics as the sample diagrammed in A. (C and D) A ⟨near midrib⟩⟨both sheath margins and one blade margin⟩ sector. Note that the sectors are located less than two lateral veins from the midrib (see C, phytomer 8 and D, phytomers 11 and 12) and mark both margins of the sheath but only one margin of the blade (see C phytomers 7 and 9; D phytomers 10 and 12). (E) Saddle sectors mark both sides of the phytomer in the blade, sheath, and node/internode. The position of the sectors alternates from closer to the midrib (i.e., phytomers 12, 14, and 16) to closer to the margin (i.e., phytomers 11, 13, 15, and 17) in successive phytomers. (F) An ⟨off midrib⟩⟨off margin⟩ sector type that is greater than two lateral veins from the midrib in phytomers 10, 12, and 14 and marks one side of the sheath, blade, and node/internode far from the margin in phytomers 9, 11, and 13. (G and H) ⟨off midrib⟩⟨off margin⟩ sectors in juvenile phytomers. Note that although the sectors fall more than two lateral veins from the midrib flank in phytomer 5, they mark both sheath margins in phytomer 6. Note also that in phytomer 6 the sectors intersect the blade more than eight lateral veins from the margin.


A
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edge than the other. Although the margins sectors passed very close to or through the middle of both the node and internode, they did not enter the leaf blade. Instead, the sheath margins sectors veered off the leaf at or below the position of the auricle (Fig. 5B). When passing through the leaf midrib, these sector types were all less than three lateral veins wide.

(Wild-type sector class 2) ⟨near midrib⟩⟨both sheath margins and one blade margin⟩ sectors. These sectors appeared close to the midrib (less than the distance of two lateral veins) of one leaf, and on both margins of the sheath but just one margin of the blade in an adjacent leaf. Nine plants with such sector types were recorded in this study (Figs. 4C, 4D, 5D, and 5E). The sectors were slightly skewed away from the center of the node and internode of their corresponding leaves, and always fell far closer to one sheath edge than the other. Invariably, the sheath sector that was located closest to the leaf edge terminated in the sheath, whereas the sector farther from the sheath edge continued into the blade (Fig. 5E). The blade sectors were very wide, spanning as many as six lateral veins, and encompassed the leaf margin.

(Wild-type sector class 3) ⟨off midrib⟩⟨off margin⟩ sectors. In fully adult leaves (L8 and on) these sectors were located more than two lateral veins away from the midrib of one leaf blade, and greater than two lateral veins from the edge of adjacent leaf blades. As observed in 22 plants and diagrammed in Fig. 4F, these sectors were restricted to only one side of the midvein in each phytomer and passed through the internode, node, sheath, and blade.

Interestingly, in juvenile leaves (leaves 1 through 6), ⟨off midrib⟩⟨off margin⟩ sectors more than two lateral veins (Fig. 4H) and three lateral veins (Fig. 4G) from the midrib of one leaf passed through *both* edges of the sheath and one edge of the blade in the adjacent leaf. In these plants, one of the sheath sectors was located close the margin edge and terminated in the sheath. However, the other sheath sector was located almost halfway between the midvein and the margin and continued into the blade.

(Wild-type sector class 4) saddle sectors. Previously described by Poethig (1986), these are relatively rare sector types which marked the node, internode, both the right and left sides (with respect to the midvein) of the sheath, *and* both sides of the blade. Only one plant in this study was found to contain a saddle sector. As shown in Figs. 4E, 5F, and 5G, the saddle sector was seen in seven successive leaves and involved the widest leaf area seen in this study (sometimes spanning greater than 7 lateral veins). The sectors were asymmetrical in the leaf, in that one side of the sector invariably passed closer to the midrib than the other side of the sector (Figs. 5F and 5G). In addition, the position of the sectors on successive leaves meandered from relatively close to the midrib, to farther out toward the margin, and then back toward the midrib (Fig. 4E). At no point did the saddle sectors encompass the edges of the lower blade. The sector could not be followed beyond node 17, since the top four leaves had not yet emerged from the surrounding leaf 17. As a consequence, the upper leaves were pale-green to white, and the identification of albino sectors in such plants is not possible.

Meristematic Sector Types Observed in ns Mutant Plants

Of approximately 500 ns mutant plants (of 2400 total plants), 18 were found that contained meristematic albino sectors. Four different classes of sector types were observed: (1) ⟨on-midrib⟩⟨margin skipping⟩; (2) ⟨near midrib⟩⟨one side leaf margin⟩; (3) ⟨off midrib⟩⟨near margin⟩, and (4) ⟨mid-leaf⟩-⟨mid-leaf⟩. Sectors observed in ns mutant plants were distinguished by several unique features not seen in wild-type sibling sectors, as detailed below.

(ns sector class 1) ⟨on midrib⟩⟨margin skipping⟩ sectors. Three separate mutant plants showed thin sectors (less than 1.5 lateral veins wide) located in the node/internode, sheath and blade midrib of one phytomer, and in the node/internode but *not* the leaf proper of the next phytomer (Figs. 6A and 7A–7C). When the sector skipped the leaf proper, the sector was observed in the phytomers above and below the skipped leaf. Therefore, in the example diagrammed in Fig. 6A, the sector did not skip the leaf proper of phytomer 11 simply because it ended at the internode of that phytomer. In contrast, the sector continued into the midrib of phytomer 12, indicating that the cells in that sectored region of the ns mutant meristem did not contribute to the leaf component of phytomer 11.

(ns sector class 2) ⟨near midrib⟩⟨one side leaf margin⟩ sectors. These sectors were located more than 1.5 lateral veins from the blade midrib of one phytomer, and were found on only one edge of the sheath and blade of the adjacent phytomer. Observed in three different plants (Figs. 6B, 6C, and 7D–7F), it is remarkable that these sectors occupied

FIG. 5. Photographs of non mutant sectored plants. (A, B, and C) An ⟨on-midrib⟩ (A) ⟨sheath margins⟩ (B and C) sector. The sector lies directly on the midrib of the leaf in A and marks both margins of the sheath near the edge of the adjacent leaf in B. Note that the margin sector ends near the auricle (arrow in B) and passes interior to the edge of the sheath as shown in C. (D and E) A ⟨near midrib⟩⟨both sheath margins and one blade margin⟩ sector marks the leaf blade less than two lateral veins from the center midrib (D), and both margins of the sheath and a wide region of the blade margin in the adjacent leaf (E). Note that the blade sector is continuous with the sheath sector located further from the edge of the leaf. (F and G) Successive leaves from a nonmutant plant with a saddle sector. The sector marks both sides of the blade and alternates from closer to the margin (F) to closer to the midrib (G) in successive leaves. b, leaf blade; s, leaf sheath.

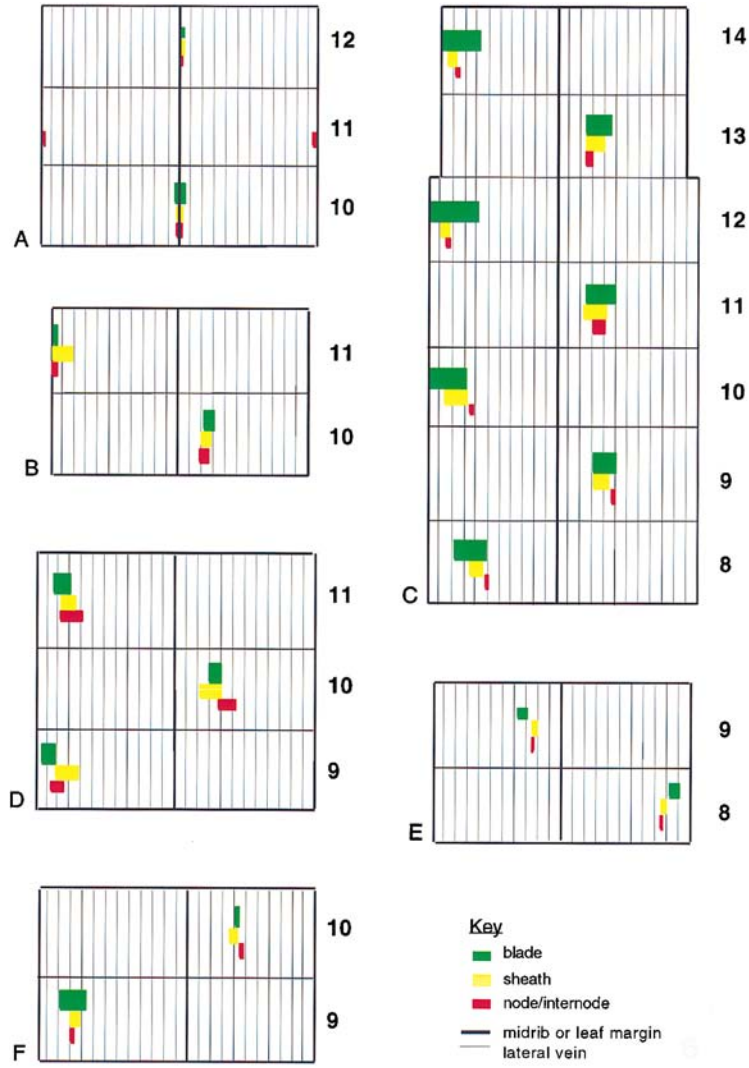
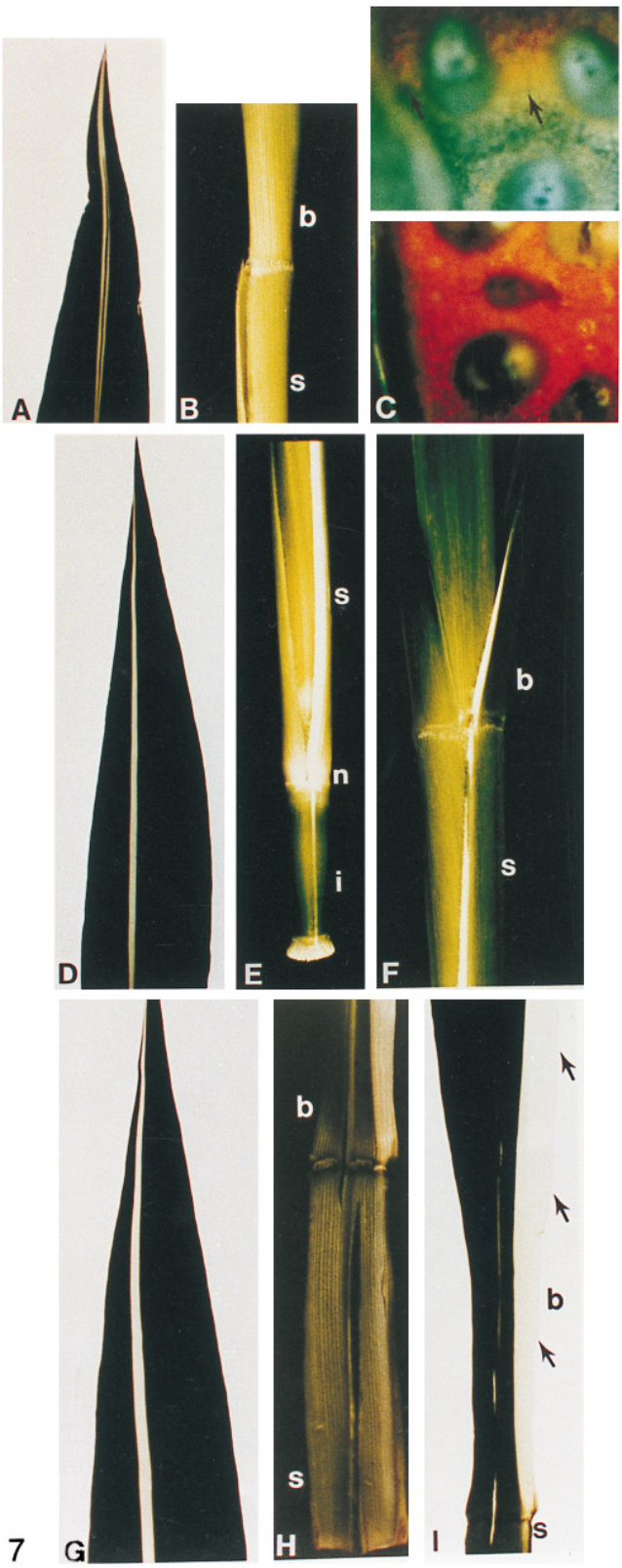


FIG. 6. Representative sector types observed in ns mutant plants. The diagrams are organized as described in the legend to Fig. 4; note that in all ns mutant phytomers the number of lateral veins are about one half that seen in nonmutant siblings. (A) An ⟨on midrib⟩⟨margin skipping⟩ sector. Note that the sector passed through the midrib region of the node/internode, sheath, and blade in phytomers 10 and 12, but passed through the margin region of the node/internode in phytomer 11 while skipping leaf 11. (B and C) ⟨near midrib⟩⟨one side leaf margin⟩ sectors. Note that the sectors passed greater than two lateral veins from the blade midrib of phytomer 10 in B and phytomers 9, 11, and 13 in C and touched the blade margin in phytomer 11 in B and phytomers 10, 12, and 14 in C. Also, the margin sectors marked only one side of the sheath. (D and E) ⟨off-midrib⟩⟨near margin⟩ sectors. Note that the sector passed at least three lateral veins from the blade midrib in phytomer 10 in D and phytomer 9 in E, but still passed relatively close to the blade margin in adjacent phytomers. (F) ⟨mid-leaf⟩⟨mid-leaf⟩ sectors. Note that these sector types passed greater than three lateral veins from the blade midrib in phytomer 10 and greater than two lateral veins from the blade margin in phytomer 9.

FIG. 7. Photographs of ns mutant sectored plants. (A–C) An ⟨on midrib⟩⟨margin skipping⟩ sector. The sector marks the center of the leaf blade on the midrib of the leaf shown in A but is not seen in the blade or sheath of the adjacent leaf shown in B. (C) Fluorescence microscopy of transverse sections through the midrib region (lower photo) and the margin region (upper photo) of the internode corresponding to the leaf shown in B. Note that the midrib region (lower photo) contains chlorophyll (red) whereas in the margin region (upper photo) the boundary of the white albino sector and the red nonsectored tissue (arrow) is apparent. Thus the sector was present in the internode margin of this phytomer, but skipped the leaf shown in B. (D–F) A ⟨near midrib⟩⟨one side leaf margin⟩ sector. This sector passed more than two lateral veins from the blade midrib of the phytomer shown in D, through one side of the sheath margin, (E) and a small portion of the blade margin (F) of the adjacent phytomer. A similar sector passing even farther from the midrib of one phytomer (G) marks one margin of the sheath near the edge (H) and a wider region of the blade margin (I) in the adjacent phytomer.



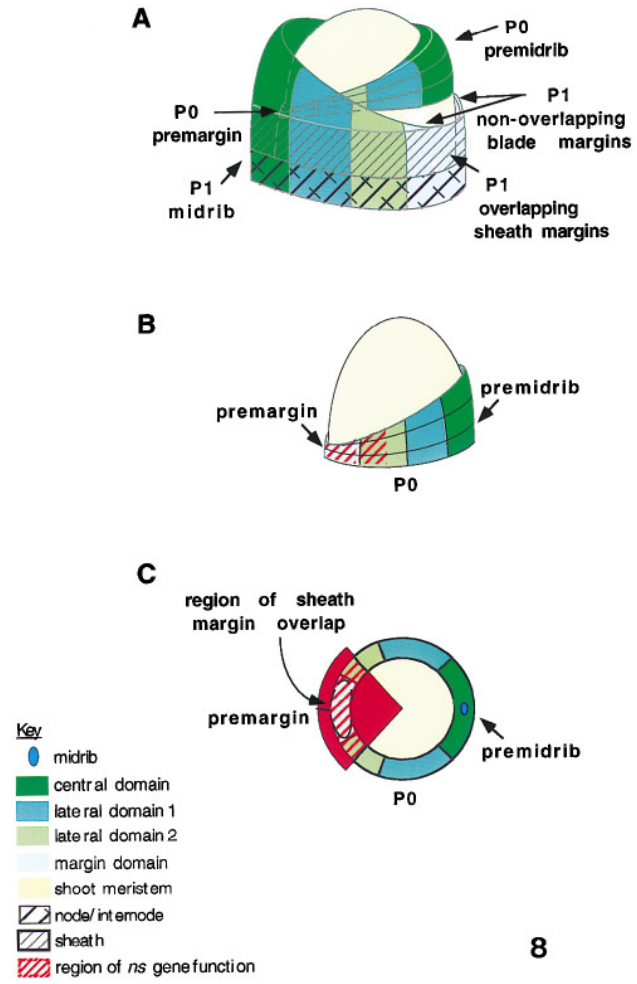


FIG. 8. Domains in the developing maize phytomer. (A) Cartoon of the maize vegetative meristem showing two successive phytomers (P1 and P0) initiated from the apex. Three hypothetical longitudinal regions are shown, corresponding to the node/internode, sheath, and blade portions of the phytomer. In addition, several color-coded, hypothetical lateral domains are illustrated. Note that in the margin of the P1 phytomer the sheath domains overlap, whereas the blade domains do not overlap. The blade domain does extend, however, into both edges of the overlapping sheath, such that a sector in these regions can mark both sides of the sheath and one side of the blade. (B) Cartoon of the ns mutant meristem and founder cells (P0). The region hatched in red represents the putative region of *Ns* gene function. Domains of the founder cell ribbon which are contained in the region of *Ns* gene function will remain uninitialized in ns mutant meristems and will be deleted from mutant leaves. (C) Cartoon of a transverse section through the ns mutant meristem founder cells. The red hatched region of *Ns* gene function is shown to include the overlapping sheath domains and portions of the adjacent lateral domains in the would-be premargin region of the developing phytomer. These regions are deleted from ns mutant phytomers.

only one edge of the sheath and never both. Sectors located on the blade margin in wild-type leaves also appeared near both margins of the sheath. In addition, margin sectors in ns mutant leaves are associated with adjacent leaf sectors located far from the midrib; this is in contrast to what is observed in wild-type plants with margin sectors. For example, a sector that passed over two lateral veins from the blade midrib of narrow sheath L10 just nicked a small edge of the margin of the next leaf (Figs. 6B, 7D, and 7E). Another sector (Figs. 6C and 7G–7I) that passed three lateral veins from the midrib of L9 occupied the blade margin just over two lateral veins wide on L10. In wild-type plants, sectors over two lateral veins from the blade midrib in L10 fell greater than seven lateral veins from the blade margin of the next leaf (Fig. 4F). Furthermore, in adult wild-type leaves margin sectors are associated with adjacent leaf sectors that are less than two lateral veins from the midrib (Figs. 4C, 4D, 5D, and 5E).

(ns sector class 3) ⟨off-midrib⟩⟨near margin⟩ sectors. Observed in three different plants, these sectors were located more than two lateral veins from the midrib of one phytomer and close to the margin edge of the blade in the adjacent phytomer (Figs. 6D and 6E). For example, in Fig. 6D, the sector is located more than three lateral veins from the blade midrib of leaf 10 (L10). The same sector appears 0.5 lateral veins from the margin of L9 and less than 1.5 lateral veins from the blade margin in L11. In contrast, sectors located more than two lateral veins from the midrib of wild-type plants are associated with adjacent leaf sectors located more than four lateral veins from the blade edge (Fig. 4F).

(4) ⟨mid-leaf⟩⟨mid-leaf⟩ sectors. These sectors passed greater than three lateral veins from the blade midrib of one phytomer, and greater than 1.5 lateral veins from the margin of the adjacent phytomer (Fig. 6F). Because they are close to neither the midrib nor the margin in any leaf, they are referred to as midleaf sectors. Nine plants were observed with this sector type.

DISCUSSION

Previous work (Poethig, 1984) has demonstrated that the founder cells of the incipient maize leaf surround the meristem such that the margins overlap, much like a key ring. The maize meristem undergoes a regular pattern of growth and cell divisions such that the fate of cells in the shoot apex (i.e., what those cells will divide into) can usually be correlated to their position on the meristem (Coe and Neuffer, 1978; Johri and Coe, 1983; Poethig *et al.*, 1986). Using clonally marked, meristematic sectors, we have exploited these features of maize leaf development to compare the fates of cells in nonmutant and ns mutant meristems. We present data from nonmutant, control plants indicating that founder cells in the maize meristem may be subdivided into lateral, developmental domains (Fig. 8A) and demonstrate which domains are contained in the founder cell overlap. Furthermore, our data reveal that the founder cells in ns mutant meristems do not overlap the apex; the margins of ns mutant phytomers develop from a meristematic domain different from that in nonmutant siblings.

Figure 9 presents cartoons depicting the positions of meristematic sectors exemplary of those described in this paper. We have shown that narrow sectors located exactly on or near the pre-midrib flank of the shoot apical meristem (the site of leaf initiation) will intersect the midvein of the blade, sheath, and node/internode of that phytomer, as well as the margins of the node/internode and sheath of the next phytomer initiated. However, these sectors did not enter the leaf blade, but marked both margins of the sheath at a position interior to the sheath edge (Figs. 5E, 4A, and 4B). These data indicate that in wild-type meristems the sheath founder cell domains surround the apex; blade founder cell domains do not overlap. Moreover, sectors induced close to the pre-midrib flank of the adult meristem intercepted successive leaves less than two lateral veins from the midrib, and near both margins of the sheath and one margin of the blade, respectively (Figs. 4C, 4D, 5D, and 5E). These sectors demonstrate that the sheath founder cell overlap extends around the meristem to include a lateral domain containing blade founder cells (see Figs. 8A and 9A). Invariably, the sector appeared closer to the sheath edge on the side of the leaf opposite the blade sector. On the other side of the leaf wherein the sector continued into the blade, the sector passed through the sheath closer to the midrib (Fig. 5E). Taken together, these data reveal the relative positions of the blade and sheath founder cell domains. As illustrated in the cartoon of the wild-type maize meristem (Fig. 8A), the margins of the blade founder cells are separated. These results indicate that the blade/sheath boundaries of the phytomer are being established at the founder cell stage, or very shortly thereafter.

Another intriguing result is that the number of lateral veins occupied by a blade sector is always greater than or equal to the number of lateral veins occupied by the same sector in the sheath (Figs. 4 and 6). Because the blade and sheath contain equal numbers of lateral veins, these data suggest that the leaf blade develops from fewer founder cells than the sheath. In turn, these data support our conclusion that the blade founder cells do not surround the shoot apex, whereas sheath founder cells do.

Saddle sectors are generated near or atop the dome of the meristem, such that the sector extends over the arched apex and down into the founder cell ribbon from two sides (Poethig *et al.*, 1986; Fig. 9A). Although they are the only sectors that mark both sides of the blade in any single phytomer (Figs. 4E, 5F, and 5E), saddle sectors *do not* demonstrate the overlap of blade founder cells. Instead, the saddle sector probably resulted from an albino clone that initiated on the slope (i.e., below the crown) of the meristem and straddled it, as shown in Fig. 9A. Because the sector was probably generated on the slope of the meristem

(see Fig. 9A), it appears to wander closer to the midrib in one leaf, and closer to the leaf margins in adjacent leaves. These sector types are rare (only one saddle-sectored plant was identified among nearly 2400 plants), which is the most likely reason such sector types were not identified among the population of ns mutant plants. One of the defining characteristics of saddle sectors is that the two sides of the sector unite to form a single sector in an upper node (Poethig *et al.*, 1986). Because leaves in the upper nodes (leaves 18–21) of this plant had not yet emerged from the surrounding lower leaves before the plant was harvested, we could not follow the sector past leaf 17. Therefore, because we did not observe the unification of the two sectors it is possible that this plant contained two, independently induced sectors instead of a saddle sector.

Furthermore, the ⟨off midrib⟩⟨off margin⟩ wild-type sectors (Fig. 4F) are also explained in terms of meristematic, founder cell domains. In this regard, a sector that is far from the midrib flank of the meristem (i.e., more than two lateral veins) will miss the overlapped sheath founder cells of the next initiated phytomer (Fig. 9A). Instead, these sectors will intersect only one margin of the sheath and pass through the blade founder cells at a position internal to the margin.

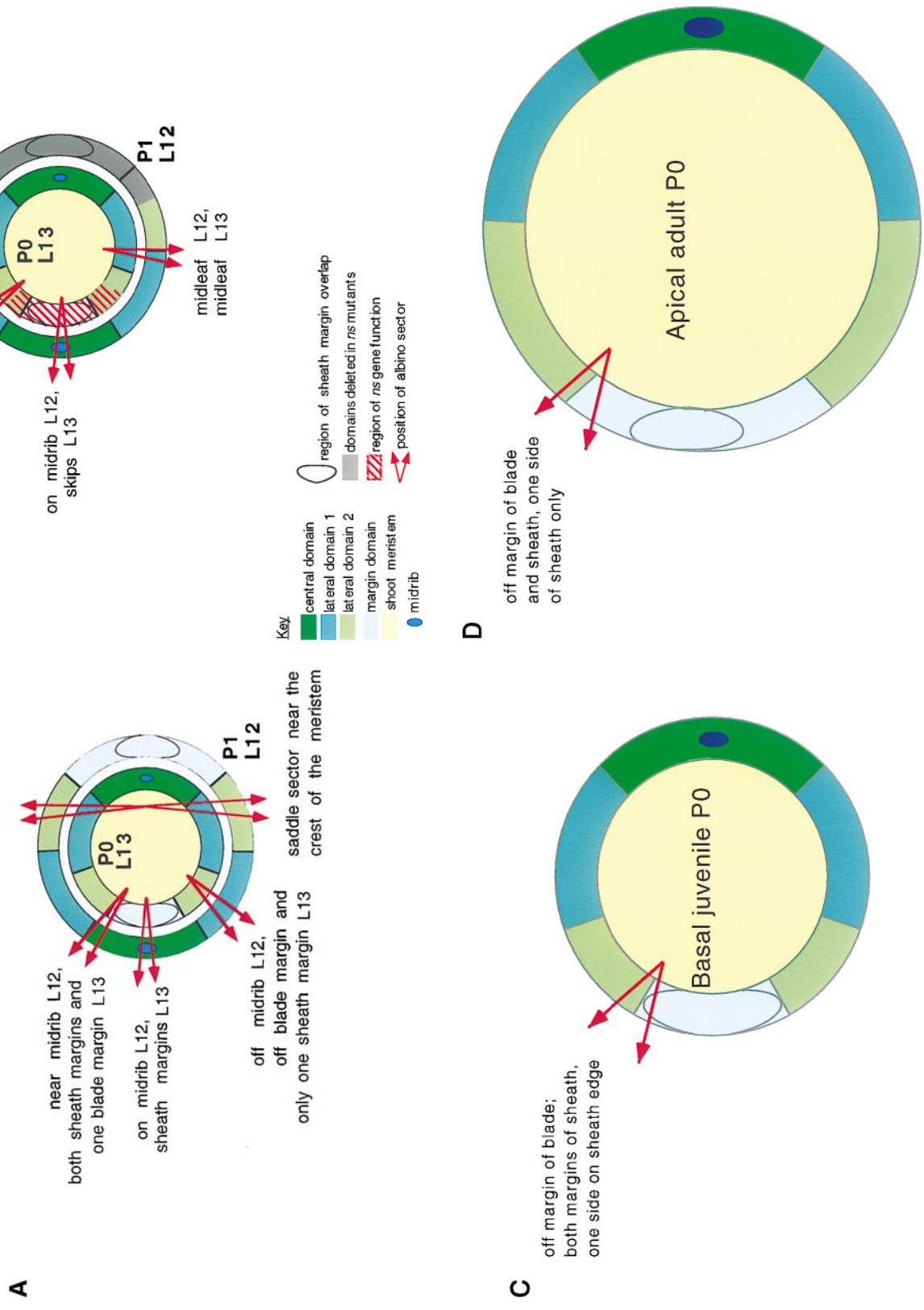
The maize meristem enlarges at every plastochron and undergoes a burst of circumferential growth during the transition from juvenile (leaves 1–7) to adult (leaves 8 and up) vegetative growth (Bassirri *et al.*, 1992). As diagrammed in Figs. 9C and 9D, this pattern of meristematic growth may permit the founder cells of juvenile leaves to overlap the smaller, juvenile-stage meristem more completely than in adult leaves, despite the smaller size of juvenile leaves. Therefore, sectors located far from the organogenic flank of the small juvenile-stage meristem may mark both margins of the sheath because juvenile-stage founder cell ribbons overlap more extensively (Fig. 9C). Our results support these conclusions. As shown in Figs. 4G and 4H, sectors located more than two lateral veins from the midrib of a juvenile leaf intercept both sheath margins in adjacent juvenile leaves. In contrast, similarly situated sectors on the larger adult-stage meristem do not intercept the sheath founder cell overlap (Fig. 9G) and therefore mark only one margin of the sheath (Fig. 4F).

The ns mutant sectored plants demonstrate fundamental differences in founder cell utilization and arrangement in ns mutant versus nonmutant plants. Whereas sectors located on the midrib of wild-type plants intercept both sheath margins of the adjacent leaf, sectors located on the midrib of ns mutants skip the adjacent leaf entirely (Figs. 6A, 7A, and 7B). These ⟨on midrib⟩⟨margin skipping⟩ sectors of ns mutants did intersect the node and internode of the skipped leaf (Fig. 7C), as well as the midribs of the phytomers adjacent to the skipped leaf (Fig. 6A). Therefore, these sectors were transmitted through the meristem and did not simply terminate before intersecting the skipped leaf. Moreover, sectors on ns mutant plants never marked both margins of the sheath (Figs. 6 and 7). Together these data indicate that, unlike wild-type siblings, the founder cells of ns mutants do not overlap the apex (Figs. 8B and 8C). In fact, the premargin flank of ns mutant meristems does not contribute to the leaf founder cell ribbon (Fig. 9B).

Sectors present on the blade edges of ns mutant, adult phytomers are associated with adjacent leaf sectors which are more than two lateral veins from the midrib (Figs. 6B, 6C, and 7D–7I). In contrast, sectors located more than two lateral veins from the blade midrib of wild-type leaves are found far from the blade margin of the next phytomer (Fig. 4F). Therefore, the edges of the ns sheath and blade founder cells (Figs. 8B, 8C, and 9B) are positioned much farther from the flank of the meristem than in nonmutant siblings (Figs. 8A and 9A). Furthermore, because leaf margin structures such as tapered edges and margin hairs are deleted from ns mutant leaves and replaced by morphological structures usually found in more laterally internal (i.e., closer to the midrib) leaf regions (Scanlon *et al.*, 1996), we suggest that the edges of ns leaves are comprised of a different lateral domain than in nonmutant siblings.

One might predict that a smaller founder cell ribbon would generate a mutant leaf that is smaller and thinner than wild type, but retains all the lateral domains seen in wild type. That is, a smaller founder cell ribbon might be expected to give rise to a proportionally smaller, yet complete mutant leaf with all domains intact. Intriguingly, the ns mutant leaf is not complete and ''shrunken,'' but is more akin to a ''cutout'' organ, with the margins removed. Furthermore, the cells which are positioned at the edges of the ns mutant leaf are not reprogrammed to assume the

FIG. 9. (A) Cartoon of a transverse section through a nonmutant shoot apex showing representative meristematic sectors which intersect leaf domains within two successive developing phytomers (P1, L12 and P0, L13). (B) Cartoon of a transverse section through a ns mutant shoot apex showing representative meristematic sectors which intersect leaf domains within two successive developing phytomers (P1, L12 and P0, L13). Sectors located in the uninitialized founder cell region of *Ns* gene function (hatched red) in the P0, L13 phytomer do not appear in the leaf, but are transmitted through the meristem into the next phytomer initiated. Note that sectors which mark the ns mutant leaf edges are much farther from the flank of the mutant meristem than leaf edge sectors seen in nonmutant siblings (A). Sectors marking the same relative position of juvenile-stage meristems (C) and adult-stage meristems (D) appear in different domains in respective phytomers. Because the juvenile meristem is much smaller than the adult-staged meristem, the founder cell ribbon surrounds the apex to a greater degree, resulting in extensive overlap of sheath margin domains. Therefore, a sector that is induced far from the marginal flank of the meristem will mark both margins of the juvenile sheath (C), whereas a similar sector will miss the sheath overlap in adult leaves (D) and therefore only mark one margin of the sheath.



developmental profiles of normal leaf margin cells. These data indicate that domain identities in the leaf may be determined by the position of founder cells in the shoot apical meristem.

We propose that the *Ns* genes function to initialize founder cells from previously naive meristematic cells in the premargin region of the meristem (outlined in red in Figs. 8B and 8C). We further suggest that other genes initialize founder cells in other regions of the apex. Our model also predicts that the specific, lateral domain identities of the developing phytomer are ascribed after founder cell initialization. Thus, the chronology of early events in phytomer determination are: (1) founder cell initialization in broad regions of the meristem, followed by (2) subdivision of these meristematic regions into more specific, lateral domain identities. This model implies that the *ns* mutations can remove differing amounts of lateral phytomer domains in various nodes, depending upon how many lateral domains will be encompassed by the uninitialized regions of the ns mutant meristem. The phenotype of ns mutant leaves and internodes is more severe in juvenile nodes and is alleviated in the upper, adult phytomers (Scanlon *et al.*, 1996). We suggested that because juvenile phytomers are made from a smaller shoot meristem than adults (Bassirri *et al.*, 1992), the juvenile-staged founder cells are more overlapped. As a consequence of this increased overlap more founder cell domains are left uninitialized in juvenile meristem than in adult meristems. This results in the deletion of more lateral domains from juvenile phytomers than from adult phytomers. Our data reveal that founder cells in juvenile-staged meristems are indeed more overlapped than adult-staged founder cells (Figs. 9C and 9D), as predicted in our model for the alleviation of the ns mutant phenotype.

The results presented here provide evidence that plant organs may be subdivided into specific domains, and that this subdivision may begin in the shoot apical meristem itself. Our results indicate that the *Ns* genes may function to initialize founder cells in a broad region on the margin flank of the meristem. Future clonal analyses employing a marker mutation that is both proximal and linked to one of the *Ns* loci will help to specify further the focus of *Ns* gene action and determine whether the narrow sheath phenotype is cell autonomous or domain autonomous.

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