INTRODUCTION

The process of compartmentalization, wherein lateral organ development proceeds via subdivision of anlagen into distinct developmental domains, is a well-established principle of animal developmental biology (reviewed by Wilkins, 1993; Lawrence, 1992). Compartments were first identified as boundaries of cell lineage restriction in the *Drosophila* wing; clonal sectors induced in the nascent wing cannot traverse from one developmental compartment into another (Garcia-Bellido et al., 1973). Compartmentalization of the *Drosophila* wing begins during early stages of imaginal disc development, before the primordial wing is elaborated. Subsequent investigations proved that animal compartments demarcate domains of differential gene expression. These domains are later sub-divided into smaller compartments, each characterized by discrete patterns of gene expression. Signalling between compartments, and de novo axis formation at compartmental boundaries, promotes the development of a three dimensional organ (Basler and Struhl, 1994; Williams et al., 1994; Tabata et al., 1995). Thus, the process of compartmentalized gene expression during *Drosophila* wing development results in a composite adult organ composed of distinct lateral, proximal-distal, and dorsiventral domains. Much less is understood about the molecular mechanisms governing organ development in plants.

At maturity, plant leaves have morphologically and functionally distinct developmental regions. In the maize leaf, for example, the ligule/auricle domain demarcates the proximal blade from the distal blade. Epidermal cell morphologies and hair types differ in the dorsal (adaxial) and ventral (abaxial) surfaces of leaves, whereas the midrib, margins and intervening vasculature make up the lateral leaf domains (Freeling and Lane, 1994; Freeling, 1992). When are these leaf domains established? Does morphogenesis of plant lateral organs proceed via the subdivision of pre-primordial developmental fields via compartmentalized gene expression? Recent investigations of plant leaf morphogenesis have begun to probe these questions.

Cell lineage analyses of plant leaves (Poethig, 1984; Dolan and Poethig, 1998; Irish and Sussex, 1992; Furner and Pumfrey, 1992) show that angiosperm leaves develop via recruitment of many (ranging from ≈30 in *Arabidopsis* to ≈250 in maize) founder-cells from the periphery of the shoot apical meristem (SAM). Founder-cell recruitment commences at the presumptive midrib region of maize leaves and gradually proceeds laterally to incorporate the initials of the leaf margins, which overlap the opposite flank of the SAM (Fig. 1). In simple-leaved angiosperms, this pattern of founder-cell recruitment is mirrored by the expression of *KNOUGHTED1*-orthologous homeobox (*KNOX*) genes in the SAM (Jackson et al., 1994; Long et al., 1996; Waites et al., 1998). *KNOX* genes are abundantly expressed in the core of the SAM and down-regulated in leaf founder-cells and leaves.
Clonal analyses clearly demonstrate that plant cell fate is determined by positional cues and not cell lineage. Therefore, if compartmentalization does occur during development of plant lateral organs, the borders of plant compartments do not designate inviolable boundaries to cell fate acquisition. Nonetheless, cell fate mapping has revealed that the fate of a leaf founder-cell (i.e. what particular leaf domain a founder-cell will eventually become) can be roughly estimated according to its position on the SAM (Poethig, 1984; Irish and Sussex, 1992; Scanlon and Freeling, 1997). Recent genetic analyses of angiosperm leaf development have generated testable models describing how cell fate is specified in plant leaves.

Correct morphological differentiation along the proximal-distal axis of plant leaves requires the precise regulation of knox genes, as demonstrated by recessive mutations in the orthologous knox regulatory loci rough sheath2 (maize; Schneeberger et al., 1998; Tsiatantis et al., 1999; Timmermans et al., 1999) and phantasy (snapdragon; Waites and Hudson, 1995; Waites et al., 1998), as well as dominant neomorphic mutations in knox genes of maize (Muehlbauer et al., 1997; reviewed by Freeling, 1992). Expression of the yabby gene family (Siegfried et al., 1999) is required to establish ventrality in Arabidopsis leaves, whereas a dominant mutation in the phabulosa gene (McDonnell and Barton, 1998) transforms ventral leaf identity into dorsal. Furthermore, analyses of mutations in the phantasy (snapdragon; Waites and Hudson, 1995) and leafbladeless1 (maize; Timmermans et al., 1998) genes have indicated that expansion along the lateral leaf axis requires the juxtapositioning of intact dorsal and ventral leaf domains.

Mutations in the narrow sheath duplicate factor pair genes cause the deletion of a lateral domain in the maize leaf that includes the margin (Fig. 2; Scanlon et al., 1996, 2000; Scanlon and Freeling, 1997, 1998). Plants homozygous for both the narrow sheath1-O (ns1-O) and narrow sheath2-O (ns2-O) mutations have narrow leaves devoid of morphological structures normally found on wild-type leaf margins, including marginal hairs and thin, tapered, leaf edges. KNOX immunolocalization studies revealed that ns mutant meristems fail to down-regulate KNOX protein accumulation in a founder-cell domain that gives rise to leaf margins in wild-type leaves (Scanlon et al., 1996). Furthermore, fate mapping of ns mutant meristems has demonstrated that a specific founder-cell domain, which normally gives rise to the would-be leaf margins, is not recruited during development of ns mutant leaves (Scanlon and Freeling, 1997). These data provide evidence that the NARROW SHEATH gene product may be required for recruitment of founder-cells in a meristemetic domain that forms the margins of maize leaves (Fig. 2).

Consequently, a model for ‘compartmentalized’ maize leaf development was proposed, in which the founder-cells of the pre-primalordial maize leaf are subdivided into at least two distinct, lateral domains (Fig. 2). One such founder-cell compartment includes the marginal domain deleted in narrow sheath mutant plants, whereas the central domain(s) includes the midrib plus all remaining lateral components of the maize leaf. Previously, sectors of dual-aneuploidy suggested that ns gene function is not required for development of the central domain in maize leaves (Scanlon et al., 2000).

In this paper, the results of a clonal analysis of NS1 function are presented. X-ray-induced, albino-marked sectors of ns1 mutant tissue were generated against a non-mutant background in order to discern the cell autonomy, tissue layer specificity, developmental timing and focus of NS1 function. The sector data demonstrate that NS1 function is localized to two discrete focal points in the internal tissue layer of the maize meristem. Loss of NS1 function during primordial stages has no discernible effect on maize leaf development. NS1 function is non-cell-autonomous within the narrow sheath domain, and promotes lateral recruitment of marginal founder-cells. Loss of NS1 function in founder cell regions marginal to the narrow sheath foci results in no phenotypic consequence, indicating that NS1 is required for initiation, but not propagation, of the founder-cell recruitment signal. These results provide further evidence for compartmentalization during pre-primalordial stages of maize leaf development.

MATERIALS AND METHODS

Genetic stocks and manipulations

The ns1-O mutation maps to the long arm of chromosome 2, approximately 16 cM distal to the albino mutation w3 (Scanlon et al., 2000). The w3 mutant seed was obtained from D. Robertson, Iowa State University, Ames, IA. Plants homozygous for the w3 mutation do not survive beyond the seedling stage; the mutation must be propagated in the heterozygous condition. Furthermore, sectors of w3 mutant leaf tissue do not alone cause disturbances in leaf morphological development (Foster et al., 1999).

In order to generate stocks useful in clonal analyses of ns1, ns mutants plants homozygous for ns1-O and ns1-O, and heterozygous for w3 (genotype W3, ns1-O/w3, ns1-O; ns2-O/n2-O as determined by examination of 1/4 white kernels segregating on self-pollinated ears) were crossed as male to non-mutant plants of the genotype: Ns1/W3, Ns1; ns2-O/ W3, ns1-O/ ns2-O (stock described in Scanlon et al., 2000). One half of the resulting progeny are expected to segregate non-mutant plants of the genotype: w3, ns1-O/W3, Ns1; ns2-O/ns2-O, in which the w3 albino mutation is linked in coupling to the ns1-O mutation, and both mutations are heterozygous (Fig. 3). Because the parental stock that was heterozygous for the w3 mutation was also homozygous for the ns1-O mutation, the w3 marker mutation will remain in coupling with the ns1-O mutation whether or not there is a meiotic crossover between the two loci. Therefore, the only possible way that X-ray-induced loss of the non-mutant W3 gene would not result in an albino sector mutant for ns1 is in the event of a somatic crossover between ns1 and w3, which would uncouple the two mutations. Since the rate of mitotic recombination in maize is vanishingly low (J. Birch, personal communication), this scenario is non-problematic. Accordingly, this crossing scheme is useful for the generation of albino-marked, ns1 mutant sectors, as described below.

Sector generation and analyses

A total of 2,937 seed, homozygous for the ns2-O mutation, and segregating heterozygosity for w3 and ns1 O in coupling (described above), were imbibed for 2 days and subsequently irradiated at 1,000 rad, 1,250 rad or 1,500 rad. The irradiation was performed by Jay Markham, radiation technician at Athens Regional Hospital, Athens, GA, utilizing cobalt 60 and average energy 1.25 MeV. Following irradiation treatment, the seed were hand-placed in damp soil at the University of Georgia Plant Sciences Farm, Oconee County, GA.

At maturity, plants containing albino clonal sectors were harvested and analyzed as described previously (Scanlon and Freeling, 1997). All sectors were either photographed or photocopied. The width and lateral position of each sector was recorded in the internode, node,
leaf sheath and blade. For sectors entering the leaf blade, the fraction of lateral veins encompassed by the sector were recorded.

Meristematic founder-cells give rise to all components of the maize phytomer, including the internode, node, leaf and bud (Poethig and Szymkowiak, 1995). Unlike the dorsiventrally flattened leaf, the internode is radially symmetrical at maturity and therefore approximates the geometry of the SAM from which it was initiated. Therefore, lateral positions of meristematic sectors initiated on the SAM were estimated by extrapolation from the boundaries of the sector as it passed through the internode (Fig. 4). For example, a sector beginning directly on the midpoint of an internode that is 4 cm in circumference and extending 5 mm in the counterclockwise direction (tabulated as midrib right, 0.0-0.5 cm) is mapped to an arc from 0 degrees to 45 degrees (out of 360) from the midrib flank of the SAM (Fig. 4).

For sectors not intersecting the internode or node, the fraction of the total number of lateral veins (measured from midrib to margin) spanned by the sector was determined as described by Scanlon and Freeling (1997), and compared to similar-sized and staged leaves (determined by leaf number, length, width and number of lateral veins) containing sectored internodes. These data were used to extrapolate the sector position in the shoot apex. Suppose, for example, a meristematic sector beginning midway between the midrib and margin flanks of the internode of a juvenile leaf, extending 12.5% of the stem circumference to the margin flank and spanning veins 10-12 of a 1/2 leaf containing 12 total veins. In addition, suppose that the non-sectored side of the ns 1/2 leaf blade contained 15 lateral veins. Using the position of the sector in the internode as a guide, the lateral position of the sector on the SAM is mapped to an arc beginning at 90° and ending 45° toward the margin flank (see Fig. 4). Accordingly, the estimated position of a second sector found on a similar-sized, non-mutant juvenile leaf that spanned veins 10 to 12 of 15 total veins and skipped the internode, would thereby be mapped to an arc extending from 90° to 45° toward the margin flank.

Histology
Shoot apices were dissected from 14-day-old maize seedlings, fixed in FAA, paraffin embedded, and sectioned at 10 μm and mounted on slides as described by Sylvester and Ruzin (1994). Images of median sectioned apices were photographed using UV fluorescence microscopy as described in the following section.

Microscopic examination of leaf tissues
Transverse, hand sections of freshly harvested maize leaves were examined via UV autofluorescence microscopy using a Zeiss Axioplan 2 (or a Zeiss Axiophot) equipped with an Attoarc HBO 100W lamp, as described by Becraft and Freeling (1994). For analyses of stomatal guard cells, adaxial and abaxial epidermal tissue layers were peeled from maize leaves as described by Harper and Freeling (1996), and analyzed using UV fluorescence microscopy. Samples were photographed with a Zeiss MC 80 DX camera.

RESULTS
Four sector phenotypes observed
Plants homozygous for the narrow sheath2-O (ns2-O) mutation and segregating for heterozygosity at the narrow sheath1-O (ns1-O) mutation in coupling with the albino mutation white3 (w3) were subjected to X-irradiation (see Materials and Methods). Previously, dosage analyses revealed that both the ns1-O and ns2-O mutations are null alleles (Scanlon et al., 2000). Therefore, random, radiation-induced breakage of the chromosome harboring the non-mutant alleles of both ns1 and w3 generated white sectors of plant tissue that were devoid of NS function. A total of 50 sectored plants, encompassing 114 leaves, were identified among grow-outs of 2,937 irradiated seedlings. Four distinct, phenotypic classes of sectored plants were observed, including: (1) narrow sheath mutant, multiple-leaf sectors; (2) non-mutant, multiple-leaf sectors; (3) non-mutant, single-leaf sectors and (4) non-mutant, multiple-leaf, marginal sectors.

Twelve sectored plants displayed narrow sheath 1/2 leaf mutant phenotypes (Table 1). All clonal sectors that yielded a narrow sheath leaf phenotype were multiple-leaf sectors, indicating that they originated in the SAM (Steffenson, 1968; Poethig, 1984). Spanning a total of 28 leaves, the twelve ns 1/2 leaf sectors produced 14 leaves that were unsectored and phenotypically normal on one margin, but exhibited the narrow sheath mutant phenotype on the opposite, albino-sectored leaf edge (Fig. 5A-E). In all cases, the ns 1/2 leaves exhibited all aspects of the ns phenotype along the sectored leaf edge, including the loss of tapered margins, marginal hairs, and reduced numbers of lateral veins (Scanlon et al., 1996; Fig. 5D,E; Table 1). Moreover, the meristematic sectors correlated with the ns 1/2 leaf phenotype invariably passed close to, but never directly on, the midvein of the adjacent leaf. This pattern of alternate leaf sectoring is observed in meristematic chimeras of distichous plants such as maize, in which successive leaves are initiated approximately 180° apart (Fig. 1; Steffenson, 1968; Scanlon and Freeling, 1997). In this way, the midrib region of one leaf is derived from the same meristematic flank as the margin domain of the adjacent leaf (Fig. 1). In summary, sectors that conferred the narrow sheath mutant phenotype were all meristematic in origin and located close to, but not on, the midrib/margin flank of the SAM.

In maize leaves, founder-cells forming the right and left
margins of the sheath (but not the blade) overlap the flank of the SAM. Thus, marginal meristematic sectors intersect both margins of the sheath (Scanlon and Freeling, 1997). In all cases where mutant sectors were identified on juvenile or mid staged leaves the sectors entered both sides of the leaf sheath, although only one side displayed the ns phenotype (Table 1). Without exception, the sector path along the wide, non-mutant leaf edge of these juvenile and mid-staged leaves never traversed the length of the sheath, but trailed off the leaf edge before reaching the auricle. Conversely, the path of the sector along the mutant leaf edge was always closer to the midrib than on the non-mutant leaf side and entered the upper leaf region (blade). Interestingly, five of the six narrow sheath 1/2 leaf phenotypes identified in adult-staged leaves contained albino sectors that marked only the mutant side of the leaf sheath and blade.

A second category of multiple-leaf sectors involved 12 plants, spanning 36 total leaves, of non-mutant leaf phenotype (Table 2; Fig. 5F,G). Although these sectors were meristematic in origin, all were located within the central domain of the leaf and did not approach the ns lateral domain (Fig. 2). Therefore, ns mutant sectors confined to the central domain do not disrupt development of the maize leaf.

The most abundant class identified among the irradiated plants in this study consisted of single-leaf sectors (Table 3; Fig. 5H). None of the 15 single-leaf sectors conditioned the ns mutant phenotype. These included seven sectors that encompassed all longitudinal components of the phytomer: the internode, node, leaf sheath and leaf blade (Sharman, 1942; Galinat, 1959). In their detailed clonal analyses of maize phytomer development, Poethig and Szymkowiak (1995) concluded that single-leaf sectors spanning all phytomer

![Image](Fig. 2. The narrow sheath mutant phenotype is a deletion of a lateral domain that includes the leaf margins. (A) Mature leaf from a narrow sheath mutant (left) and a non-mutant sibling (right) plant. (B-D) A model for NARROW SHEATH function during recruitment of phytomer (internode, leaf sheath and blade) founder-cells in a marginal domain. (B) Founder-cells are recruited from the maize shoot apical meristem (SAM) beginning in the midrib domain and progressing toward the opposite flank, which includes the overlapping founder-cells that give rise to the right and left margins of the leaf sheath. (C) Model of the narrow sheath leaf primordium, which contains the central domain of the phytomer but lacks the marginal domains shown in yellow on the non-mutant leaf primordium (D). B, leaf blade; S, leaf sheath; lg, ligule; md, leaf midrib; mg, leaf margin.)

![Image](Fig. 3. Generation of narrow sheath1 mutant, albino sectors. Cartoon of a maize cell heterozygous for the ns1 and w3 mutations in coupling on the long arm of chromosome 2. X-ray-induced breakage of the non-mutant chromosome between the centromere (black rectangles) and the non-mutant W3 allele will engender the loss of the wild-type Ns1 and W3 alleles in mitotic progeny, exposing the ns1 mutation in an albino-marked sector.)
Clonal analysis of NARROW SHEATH1 components probably result from clonal mosaicism induced at the founder-cell stage of development. Sectors induced during progressively later stages of development, during which the phytomer components become clonally separate, are confined to successively smaller intervals of the mature phytomer (Poethig and Szymkowiak, 1995). Twelve of the fifteen sectors spanning a single leaf were found on juvenile leaves (leaves no. 1-7); these basal-most leaves are formed during maize embryogenesis and shortly after germination (Sharman, 1942). The remaining three single-leaf sectors marked leaf no. 10, which develops soon after seed germination. No single-leaf sectors were identified on leaf no. 11 or beyond. These later-staged leaves are formed from the SAM several days after germination, therefore, seedlings irradiated 2 days after germination are not expected to produce single-leaf mosaics (i.e. sectors induced during founder cell stages or later) in late-staged leaves (Steffenson, 1968; Poethig, 1984; Poethig and Szymkowiak, 1995).

Notably, no single-leaf sector identified in this study resulted in the ns mutant leaf phenotype. These included single leaf sectors that encompassed the leaf margin and spanned many lateral veins (Table 3 and Fig. 7). For example, the single-leaf sector shown in Fig. 5H spanned 6 lateral veins astride the edge of a leaf blade that contained 13 lateral veins from midrib to one margin, yet developed a fully non-mutant leaf margin (Fig. 5H,I). Moreover, this sector did not enter all longitudinal components of the phytomer, but marked only one sheath margin and missed the internode entirely (Fig. 5H). In maize leaves, the founder-cells of the right and left margins of the sheath (but not the blade) overlap the flank of the SAM. Thus, marginal meristematic sectors intersect both margins of the sheath (Scanlon and Freeling, 1997). Taken together, these data...
Fig. 5
Fig. 5. Sector phenotypes. (A-C) Narrow sheath 1/2 leaf phenotypes generated by marginal, multiple-leaf sectors (arrows) result in a marked decrease in width of the leaf blade (b) and sheath (s). (A) Sector 7. (B) Sector 2. (C) Sector 30. (D) Fluorescence micrograph of a transverse section of the leaf margins at the non-sectored edge of the narrow sheath 1/2 leaf shown in A. The epidermal cells are mostly non-photosynthetic (green-blue), whereas the chlorophyll mesophyll (m) cells fluoresce red. The lateral veins (iv) do not abut the tapered, non-photosynthetic leaf tip. (E) Fluorescence micrograph of the albino-sectored edge of the narrow sheath 1/2 leaf shown in A. The mesophyll layer is unpigmented, whereas the leaf tip is blunt and much thicker than in non-mutant leaf edges. The presence of lateral veins adjacent to the leaf edge is typical of narrow sheath mutant leaves. (F) Sector 27, leaf 6; the sector (arrow) is in the central leaf domain and the leaf width is normal. (G) Micrograph of a transverse leaf section of sector 27, leaf 6. The sector (arrow) is astride the midvein, in the central leaf domain. (H) Sector 3 (arrow) spanned the right margin of both the blade and sheath of a single leaf, but did not enter the left sheath margin or the internode. (I) Micrograph of sector 3 reveals non-mutant morphology at the leaf blade margin. At this point in the blade, the sector ends one lateral vein (arrow) from the leaf edge. (J-L) Non-mutant leaf phenotypes found on multiple-leaf, margin sectors. (J) Sector 15 (arrows) passed near the midrib of the blade (b1) and sheath (s1) of a lower leaf; on the blade (b2) and sheath (s2) margins of the middle leaf; and continued near the midrib of the upper leaf (s3). (K) Sector 32 (arrows) passed near both edges of the sheath (s1) and one edge of the blade (b1) in the lower leaf, and continued near the midrib of the next leaf (s2). (L) Sector 48 passed through both margins of the shear (arrows) and skipped the blade of leaf 11. (M) Micrograph of the non-sectored, left edge of the blade of the middle leaf shown in J. (N) Micrograph of the sectored, right edge of the middle leaf shown in J. Note that despite the albino tissue, the margin morphology of the sectored leaf shown in N is normal. The fluorescence of the chlorophyllous tissues in D and M appears a different shade of red to those shown in G and I because they were photographed on different microscopes with slightly different filters. Md, midrib; ab, abaxial epidermis; ad, adaxial epidermis; iv, intermediate vein; lg, ligule; c, ear.

reveal that the margin sector shown in Fig. 5H (which marks one sheath margin only) was induced after the founder-cell stage of development, and not in the founder-cells or SAM. These parameters were used to estimate that, of the 15 single-leaf sectors observed in this study, seven were induced at the founder cell stage and eight were post-founder-cell staged (Table 3). Moreover, six of the 12 single-leaf sectors found on juvenile leaves, and one of the three mid-staged chimeras, were judged to be founder-cell sectors.

Perhaps the most interesting and informative ns clonal mosaic plants identified in this analysis are the multiple-leaf, non-mutant margin sector types (Table 4; Fig. 5J-N). Including 11 chimeras that encompassed 35 total leaves, this sector class contained 20 leaves exhibiting albino tissue devoid of NS function on one blade margin and/or both sheath margins. As shown in Fig. 5J, loss of NS1 function in the right blade margin and both the right and left margins of the sheath nonetheless resulted in normal development of all leaf lateral domains.

Table 1. Narrow sheath meristematic sectors

<table>
<thead>
<tr>
<th>Sector (leaf)*</th>
<th>Leaf length</th>
<th>Lateral veins‡</th>
<th>Leaf stage§</th>
<th>Leaf phenotype</th>
<th>Internode girth</th>
<th>Sector position in internode¶</th>
<th>Tissue layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (L5)</td>
<td>37 cm</td>
<td>8 veins</td>
<td>J</td>
<td>wild type</td>
<td>5.8 cm</td>
<td>midrib right 5 mm-6mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>1 (L6)</td>
<td>47 cm</td>
<td>7-13</td>
<td>J</td>
<td>narrow sheath 1/2 leaf</td>
<td>6.8 cm</td>
<td>margin left 5 mm-7 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>2 (L6)</td>
<td>42 cm</td>
<td>8-15</td>
<td>J</td>
<td>narrow sheath 1/2 leaf</td>
<td>6 cm</td>
<td>margin left 5 mm-8 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>2 (L7)</td>
<td>54.5 cm</td>
<td>18</td>
<td>J</td>
<td>wide type</td>
<td>6.8 cm</td>
<td>midrib right 1 mm-2 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>4 (L4)</td>
<td>51 cm</td>
<td>14</td>
<td>J</td>
<td>wild type</td>
<td>6.2 cm</td>
<td>midrib left 6 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>4 (L5)</td>
<td>58 cm</td>
<td>9-13</td>
<td>J</td>
<td>narrow sheath 1/2 leaf</td>
<td>6.9 cm</td>
<td>margin right 5 mm-6 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>7 (L14)</td>
<td>67 cm</td>
<td>18</td>
<td>M-A</td>
<td>wild type</td>
<td>5.8 cm</td>
<td>midrib left 9 mm-10 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>7 (L15)</td>
<td>66 cm</td>
<td>17-14</td>
<td>A</td>
<td>narrow sheath 1/2 leaf</td>
<td>5 cm</td>
<td>margin right 1 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>11 (L4)</td>
<td>71 cm</td>
<td>17</td>
<td>M</td>
<td>wide leaf</td>
<td>5.2 cm</td>
<td>midrib right 2 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>11 (L5)</td>
<td>74 cm</td>
<td>12-18</td>
<td>M</td>
<td>narrow sheath 1/2 leaf</td>
<td>5 cm</td>
<td>margin left 5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>12 (L6)</td>
<td>60 cm</td>
<td>13-18</td>
<td>M</td>
<td>narrow sheath 1/2 leaf</td>
<td>7.1 cm</td>
<td>margin right 7 mm-9 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>12 (L7)</td>
<td>66 cm</td>
<td>20</td>
<td>M</td>
<td>wild type</td>
<td>6.5 cm</td>
<td>midrib left 5 mm</td>
<td>ND (diffuse)</td>
</tr>
<tr>
<td>16 (L8)</td>
<td>68 cm</td>
<td>12-21</td>
<td>M</td>
<td>narrow sheath 1/2 leaf</td>
<td>4.5 cm</td>
<td>margin right 5 mm-8 mm</td>
<td>L2</td>
</tr>
<tr>
<td>16 (9)</td>
<td>65 cm</td>
<td>18</td>
<td>M</td>
<td>wild type</td>
<td>3.2 cm</td>
<td>midrib left 2 mm-4 mm</td>
<td>L2</td>
</tr>
<tr>
<td>17 (8)</td>
<td>62 cm</td>
<td>12-20</td>
<td>M</td>
<td>narrow sheath 1/2 leaf</td>
<td>4.8 cm</td>
<td>margin left 5 mm-6 mm</td>
<td>L2</td>
</tr>
<tr>
<td>17 (9)</td>
<td>ND</td>
<td>ND</td>
<td>M</td>
<td>wild type</td>
<td>3.5 cm</td>
<td>midrib right 7 mm-8 mm</td>
<td>ND</td>
</tr>
<tr>
<td>18 (8)</td>
<td>59 cm</td>
<td>ND</td>
<td>A</td>
<td>narrow sheath 1/2 leaf</td>
<td>2.1 cm</td>
<td>margin left 5.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>18 (9)</td>
<td>53 cm</td>
<td>14</td>
<td>A</td>
<td>wild type</td>
<td>1.9 cm</td>
<td>midrib right 2 mm-2.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>18 (10)</td>
<td>50 cm</td>
<td>6-13</td>
<td>A</td>
<td>narrow sheath 1/2 leaf</td>
<td>1.5 cm</td>
<td>margin left 2.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>29 (12)</td>
<td>92 cm</td>
<td>23</td>
<td>M</td>
<td>wild type</td>
<td>6.4 cm</td>
<td>midrib left 2 mm</td>
<td>L2</td>
</tr>
<tr>
<td>29 (13)</td>
<td>88 cm</td>
<td>18-24</td>
<td>M</td>
<td>narrow sheath 1/2 leaf</td>
<td>5.9 cm</td>
<td>margin right 5 mm-6 mm</td>
<td>L2</td>
</tr>
<tr>
<td>30 (8)</td>
<td>51 cm</td>
<td>10-13</td>
<td>A</td>
<td>narrow sheath 1/2 leaf</td>
<td>2.4 cm</td>
<td>margin right 4 mm-5.5 mm</td>
<td>L2</td>
</tr>
<tr>
<td>30 (9)</td>
<td>48.5 cm</td>
<td>11</td>
<td>A</td>
<td>wild type</td>
<td>1.8 cm</td>
<td>midrib left 2 mm-4 mm</td>
<td>L2</td>
</tr>
<tr>
<td>30 (10)</td>
<td>46 cm</td>
<td>6-9</td>
<td>A</td>
<td>narrow sheath 1/2 leaf</td>
<td>1.6 cm</td>
<td>margin right 3 mm-4 mm</td>
<td>L2</td>
</tr>
<tr>
<td>33 (10)</td>
<td>65 cm</td>
<td>15</td>
<td>A</td>
<td>wild type</td>
<td>2.9 cm</td>
<td>midrib left 3 mm-5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>33 (11)</td>
<td>62 cm</td>
<td>13</td>
<td>A</td>
<td>wild type</td>
<td>2.0 cm</td>
<td>midrib right 3 mm-3.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>33 (12)</td>
<td>56 cm</td>
<td>7-12</td>
<td>A</td>
<td>narrow sheath 1/2 leaf</td>
<td>1.6 cm</td>
<td>margin left 3 mm-5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>33 (13)</td>
<td>40 cm</td>
<td>9</td>
<td>A</td>
<td>wild type</td>
<td>1.6 cm</td>
<td>midrib right 4 mm-5 mm</td>
<td>L1-L2</td>
</tr>
</tbody>
</table>

*Leaf numbers were counted at plant maturity; the leaf closest to the base was designated leaf 1 (L1).
‡The number of lateral veins per half-leaf with respect to the midvein. In leaves exhibiting the narrow sheath 1/2 leaf phenotype the number of lateral veins on the narrow side of the leaf are appear first; the number of veins on the wide side of the leaf are listed following the hyphen.
§Basal leaves represented as juvenile (J); middle phase leaves represented as middle (M); apical leaves represented as adult (A).
¶Sector position in the internode measured as described in Materials and Methods.

(Continued)
Fig. 6. Tissue layer specificity of ns1 mutant sectors. (A) Fluorescence micrograph of epidermal tissue peeled from sector 22, which contained an albino sector in both the L1- and L2-derived tissue layers (Table 1). Note the absence of chlorophyll in stomatal guard cells (arrows), which are the only epidermal cell types capable of accumulating chlorophyll. (B) Epidermal tissue peeled from ns1/2 leaf sector 30 reveals chlorophyll pigmentation (red fluorescence; seen as yellow over the green) in epidermal guard cells, indicating that the sector did not enter the epidermal tissues. The double arrowheads denote the sector boundary, which contains L2-derived as well as L1-derived tissues (i.e. unpeeled). (C) Transverse section through leaf tissue of sector 26. Note that the sector (light blue fluorescent tissue) does not include all internal tissue layers; the abaxial mesophyll (arrowheads) is non-sectored. lv, lateral vein; ab, abaxial epidermis; ad, adaxial epidermis; iv, intermediate vein.

Table 2. Wild-type meristematic sectors

<table>
<thead>
<tr>
<th>Sector (leaf)*</th>
<th>Leaf length</th>
<th>Lateral veins‡</th>
<th>Leaf stage§</th>
<th>Leaf phenotype</th>
<th>Internode girth</th>
<th>Sector position in internode¶</th>
<th>Tissue layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (L4)</td>
<td>47 cm</td>
<td>13</td>
<td>J</td>
<td>wild type</td>
<td>4.7 cm</td>
<td>margin left 1.5 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>5 (L5)</td>
<td>55 cm</td>
<td>16</td>
<td>J</td>
<td>wild type</td>
<td>4.8 cm</td>
<td>midrib right 4 mm-5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>6 (L5)</td>
<td>54 cm</td>
<td>15</td>
<td>J</td>
<td>wild type</td>
<td>7 cm</td>
<td>midrib right 1.6 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>6 (L6)</td>
<td>63.5 cm</td>
<td>15</td>
<td>J</td>
<td>wild type</td>
<td>7.4 cm</td>
<td>margin left 1.5 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>6 (L7)</td>
<td>70 cm</td>
<td>20</td>
<td>M</td>
<td>wild type</td>
<td>7.3 cm</td>
<td>midrib right 1.2 cm-1.4 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>20 (L10)</td>
<td>63 cm</td>
<td>22</td>
<td>M</td>
<td>wild type</td>
<td>4.7 cm</td>
<td>midrib left 1 cm-1.1 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>20 (L11)</td>
<td>65 cm</td>
<td>20</td>
<td>M</td>
<td>wild type</td>
<td>4.6 cm</td>
<td>margin right 1.1 cm-1.2 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>20 (L12)</td>
<td>62.5 cm</td>
<td>18</td>
<td>M</td>
<td>wild type</td>
<td>4 cm</td>
<td>midrib left 8 mm-8.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>21 (L12)</td>
<td>49 cm</td>
<td>12</td>
<td>A</td>
<td>wild type</td>
<td>2.9 cm</td>
<td>margin right 6 mm-7.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>21 (L13)</td>
<td>38 cm</td>
<td>9</td>
<td>A</td>
<td>wild type</td>
<td>2.3 cm</td>
<td>midrib left 5 mm-7 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>28 (L8)</td>
<td>73 cm</td>
<td>18</td>
<td>M</td>
<td>wild type</td>
<td>5.4 cm</td>
<td>midrib left 1 cm-1.2 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>28 (L9)</td>
<td>72 cm</td>
<td>21</td>
<td>M</td>
<td>wild type</td>
<td>4.8 cm</td>
<td>midrib right 8 mm-9 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>34 (L10)</td>
<td>69 cm</td>
<td>19</td>
<td>M</td>
<td>wild type</td>
<td>4.3 cm</td>
<td>margin left 1.1 cm</td>
<td>L2</td>
</tr>
<tr>
<td>34 (L11)</td>
<td>61 cm</td>
<td>17</td>
<td>M</td>
<td>wild type</td>
<td>3.5 cm</td>
<td>midrib right 3 mm-6 mm</td>
<td>L2</td>
</tr>
<tr>
<td>41 (L9)</td>
<td>68 cm</td>
<td>17</td>
<td>M</td>
<td>wild type</td>
<td>4.9 cm</td>
<td>midrib left 1 cm-1.2 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>41 (L10)</td>
<td>66.5 cm</td>
<td>19</td>
<td>M</td>
<td>wild type</td>
<td>4.3 cm</td>
<td>margin right 1 cm-1.2 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>41 (L11)</td>
<td>63.5 cm</td>
<td>16</td>
<td>M-A</td>
<td>wild type</td>
<td>3.2 cm</td>
<td>midrib left 6 mm-8 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>41 (L12)</td>
<td>60 cm</td>
<td>12</td>
<td>A</td>
<td>wild type</td>
<td>2.5 cm</td>
<td>margin right 5 mm-8 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>41 (L13)</td>
<td>51.5 cm</td>
<td>10</td>
<td>A</td>
<td>wild type</td>
<td>1.9 cm</td>
<td>midrib left 5 mm-6 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>42 (L8)</td>
<td>73.5 cm</td>
<td>21</td>
<td>M</td>
<td>wild type</td>
<td>5.3 cm</td>
<td>midrib left 1.7 cm-1.8 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>42 (L9)</td>
<td>71 cm</td>
<td>21</td>
<td>M</td>
<td>wild type</td>
<td>4.6 cm</td>
<td>midrib right 5 mm-7 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>42 (L10)</td>
<td>67 cm</td>
<td>18</td>
<td>M</td>
<td>wild type</td>
<td>4.0 cm</td>
<td>midrib left 1.4 cm-1.5 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>44 (L10)</td>
<td>64 cm</td>
<td>18</td>
<td>M</td>
<td>wild type</td>
<td>5.4 cm</td>
<td>margin right 4 mm-5 mm</td>
<td>L1-1 partial L2</td>
</tr>
<tr>
<td>44 (L11)</td>
<td>63 cm</td>
<td>15</td>
<td>M-A</td>
<td>wild type</td>
<td>2.9 cm</td>
<td>midrib left 2 mm-3 mm</td>
<td>L1-1 partial L2</td>
</tr>
<tr>
<td>44 (L12)</td>
<td>56.5 cm</td>
<td>14</td>
<td>M-A</td>
<td>wild type</td>
<td>2.5 cm</td>
<td>margin right 3 mm-4 mm</td>
<td>L1-1 partial L2</td>
</tr>
<tr>
<td>44 (L13)</td>
<td>53 cm</td>
<td>10</td>
<td>A</td>
<td>wild type</td>
<td>1.9 cm</td>
<td>midrib left 3 mm-4 mm</td>
<td>L1-1 partial L2</td>
</tr>
<tr>
<td>45 (L6)</td>
<td>73 cm</td>
<td>21</td>
<td>M</td>
<td>wild type</td>
<td>4.9 cm</td>
<td>margin right 8 mm-12 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>45 (L7)</td>
<td>70 cm</td>
<td>16</td>
<td>M</td>
<td>wild type</td>
<td>3.8 cm</td>
<td>midrib left 5 mm-5.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>45 (L8)</td>
<td>63 cm</td>
<td>16</td>
<td>M</td>
<td>wild type</td>
<td>3.3 cm</td>
<td>margin right 6 mm-6.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>45 (L9)</td>
<td>55 cm</td>
<td>15</td>
<td>A</td>
<td>wild type</td>
<td>2.5 cm</td>
<td>midrib left 5mm-7 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>46 (L8)</td>
<td>75 cm</td>
<td>21</td>
<td>M</td>
<td>wild type</td>
<td>6.5 cm</td>
<td>midrib left 1.5 cm-1.8 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>46 (L9)</td>
<td>74 cm</td>
<td>20</td>
<td>M</td>
<td>wild type</td>
<td>5.2 cm</td>
<td>midrib right 1.4 cm-1.7 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>46 (L10)</td>
<td>73 cm</td>
<td>20</td>
<td>M</td>
<td>wild type</td>
<td>4.15 cm</td>
<td>midrib left 8 mm-11 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>46 (L11)</td>
<td>69 cm</td>
<td>17</td>
<td>M</td>
<td>wild type</td>
<td>3.6 cm</td>
<td>midrib right 1 cm-1.1 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>47 (L7)</td>
<td>73 cm</td>
<td>20</td>
<td>M</td>
<td>wild type</td>
<td>8.4 cm</td>
<td>midrib right 1.2 cm-1.4 cm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>47 (L8)</td>
<td>78.7 cm</td>
<td>24</td>
<td>M</td>
<td>wild type</td>
<td>8.1 cm</td>
<td>margin left 1.1 cm-1.8 cm</td>
<td>L1-L2</td>
</tr>
</tbody>
</table>

*Leaf numbers were counted at plant maturity; the leaf closest to the base was designated leaf 1 (L1).
‡The number of lateral veins per half-leaf with respect to the midvein.
§Basal leaves represented as juvenile (J); middle phase leaves represented as middle (M); apical leaves represented as adult (A).
¶Sector position in the internode measured as described in Materials and Methods.
Key:
domains

midrib
central
narrow sheath
meristem proper

sector types

mutant meristematic
non-mutant meristematic
post-founder cell
founder cell
narrow sheath focus
dashed line = partial L2 layer

Fig. 7. NS1 function is localized to two focal points in meristematic founder-cells. The ns1 mutant, albino sectors in this study were mapped to lateral positions of the maize SAM, as described in the text and Fig. 4. The sectors were subdivided into those intersecting (A) basal phytomers, (B) mid-staged phytomers and (C) apical phytomers of the maize plant. As shown in the model, the estimated positions of meristematic, ns mutant 1/2 sectors (red lines) occur in two regions near the beginning of the putative marginal domains of the maize founder cells. The only non-mutant sectors that span the ns focal points are those induced either after the founder-cell stage (black lines in A), or meristematic sectors that did not include all internal tissue layers (dashed lines in B and C). Only one sectored phytomer, labeled 20-8 (C), did not conform to this model of NS1 function at two founder-cell foci. As the plant grows, the lateral position of the NS1 foci recede from the marginal flank toward the midrib flank of the SAM. See text for further explanation.
Despite the mutant sectors, leaf width and margin morphology were unaffected (Fig. 5J-L), and included the elaboration of tapered sheath edges, marginal hairs and normal blade margins (Fig. 5M,N). All these sector types were meristematic (as evidenced by their multiple-leaf phenotype), and passed either directly on, or close to, the midrib of the adjacent leaf (Fig. 5J,K). These data reveal that the multiple-leaf, non-mutant marginal sectors were induced directly on or near the midrib/margin flank of the SAM (Fig. 1; Scanlon and Freeling, 1997).

**NARROW SHEATH1 function is localized to inner tissue layers**

Both the maize epidermis and the non-chlorophyllic leaf edges (Fig. 5D) are derived from the L1 or tunica layer of the SAM (Fig. 1; Poethig and Szymkowiak, 1995). The remaining internal tissues of the leaf, including the mesophyll and vasculature, are derived from the L2 (corpus) meristematic layer. In order to monitor the tissue layer specificity of each sectored plant, chlorophyll-autofluorescence microscopy of maize leaves was performed (described in Materials and Methods). Of the 50 total sectors described in this study, 32 occupied L1- to L2-derived tissue layers (Tables 1-4; Fig. 6A). Several L1-L2 chimeras exhibited incomplete albinoism in the leaf mesophyll, localized to short stretches at one sector border (Fig. 6B). However, only nine sectors were characterized as partial L2; the internal layers of these plants were incompletely albino throughout the lateral extent of the individual sectors (Fig. 6C).

None of the 12 narrow sheath 1/2-leaf sectors were partial L2-derivatives (Table 1). In all cases the ns 1/2 leaf plants displayed fully albino, internal tissue at the mutant leaf edge (Fig. 5E). Notably, three mutant sectors were confined to L2-derived tissues only, and contained the non-mutant Narrow sheath1 gene in the epidermal layer (Fig. 6B). Therefore, NARROW SHEATH1 function in epidermal tissue is insufficient for normal maize leaf development. The remaining nine ns mutant leaf sectors were derived from both L1 and L2 layers of the SAM (Table 1).

Sixteen of 23 non-mutant, multiple-leaf sectors were contained in both L1- and L2-derived leaf tissues (Tables 2, 4). In contrast, over one-half of the single-leaf sectors did not include all tissue layers (Table 3), including two L2 sectors, two L1-partial L2 chimeras, and four partial L2 sectors.

**DISCUSSION**

One problem inherent to plant clonal analyses is the difficulty in estimating the initial location of a sector that is induced on

### Table 3. Single leaf sectors

<table>
<thead>
<tr>
<th>Sector (leaf)*</th>
<th>Leaf length</th>
<th>Leaf stage ‡</th>
<th>Tissue layer</th>
<th>Longitudinal sector position§</th>
<th>Lateral sector position¶</th>
<th>Timing of sector initiation**</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (3)</td>
<td>37 cm</td>
<td>J</td>
<td>L1-L2</td>
<td>right margin, one side sheath; veins 6-12/13.</td>
<td>post-founder cell;</td>
<td></td>
</tr>
<tr>
<td>9 (5)</td>
<td>45.2 cm</td>
<td>J</td>
<td>L1-L2</td>
<td>left margin, both sides sheath; left margin; veins 12.5-13/15.</td>
<td>founder cell;</td>
<td></td>
</tr>
<tr>
<td>10 (6)</td>
<td>56.5 cm</td>
<td>J</td>
<td>L2</td>
<td>right margin; both sides sheath; right margin, one side sheath; 4-6 mm/7.1 cm internode.</td>
<td>post-founder cell;</td>
<td></td>
</tr>
<tr>
<td>13 (6)</td>
<td>57 cm</td>
<td>J</td>
<td>L1-L2</td>
<td>upper I; S; right midrib; 1.5-2.5 mm/8.3 cm internode.</td>
<td>founder cell</td>
<td></td>
</tr>
<tr>
<td>14 (1)</td>
<td>51 cm</td>
<td>J</td>
<td>L1-partial L2</td>
<td>I; B; right midrib; 1-1.5 mm/4 cm internode.</td>
<td>founder cell</td>
<td></td>
</tr>
<tr>
<td>23 (10)</td>
<td>71 cm</td>
<td>M</td>
<td>L1-L2</td>
<td>I; S; right midrib; 1-1.5 mm/4 cm internode.</td>
<td>founder cell</td>
<td></td>
</tr>
<tr>
<td>24 (7)</td>
<td>65 cm</td>
<td>J</td>
<td>L2</td>
<td>upper S; B; left margin; veins 12-14.5/16.</td>
<td>post-founder cell;</td>
<td></td>
</tr>
<tr>
<td>25 (6)</td>
<td>56 cm</td>
<td>J</td>
<td>L1-L2</td>
<td>N; B and S; right margin, one side sheath; 2-3 mm/6.9 cm internode.</td>
<td>founder or early primordial stage</td>
<td></td>
</tr>
<tr>
<td>36 (7)</td>
<td>71 cm</td>
<td>J</td>
<td>L2 partial</td>
<td>upper I; S; B; right margin; one side sheath; 2-3 mm/6.9 cm internode.</td>
<td>post-founder cell;</td>
<td></td>
</tr>
<tr>
<td>37 (5)</td>
<td>62.5 cm</td>
<td>J</td>
<td>L2</td>
<td>partial; upper S; Bright margin veins 12-14.5/17</td>
<td>post-founder cell;</td>
<td></td>
</tr>
<tr>
<td>38 (7)</td>
<td>64 cm</td>
<td>J</td>
<td>L1- partial L2</td>
<td>upper I; S; Bright margin, one side sheath; 1.3-1.4 cm/6.1 cm internode.</td>
<td>post-founder cell;</td>
<td></td>
</tr>
<tr>
<td>39 (10)</td>
<td>72 cm</td>
<td>M</td>
<td>L2 partial</td>
<td>S; B; left margin, one sheath; veins 15.5-16/17.</td>
<td>post-founder cell;</td>
<td></td>
</tr>
<tr>
<td>40 (8)</td>
<td>81 cm</td>
<td>J</td>
<td>L2 partial</td>
<td>I; B; S; left margin, one side sheath; 8-9 mm/5 cm internode.</td>
<td>founder cell</td>
<td></td>
</tr>
<tr>
<td>49 (10)</td>
<td>75 cm</td>
<td>M</td>
<td>L1-L2</td>
<td>N; B; S; right margin, one side sheath; 3-5 mm/5.1 cm node.</td>
<td>post-founder cell;</td>
<td></td>
</tr>
<tr>
<td>50 (5)</td>
<td>57 cm</td>
<td>J</td>
<td>L1-L2</td>
<td>I; S; B; left margin, both sides sheath 2-3 mm/5 cm internode.</td>
<td>founder cell</td>
<td></td>
</tr>
</tbody>
</table>

*Leaf numbers were counted at plant maturity; the leaf closest to the base was designated Leaf 1 (L1).

‡Basal leaves represented as juvenile (J); middle phase leaves represented as middle (M); apical leaves represented as adult (A).

§I, internode; N, node; S, leaf sheath; B, leaf blade.

¶For sectors passing through the internode and/or node, the sector position is designated by listing the boundaries of the sector as measured from the midpoint of the internode margin or internode midrib divided by the internode circumference. For sectors not present in the internode or node, the fraction of the total number of lateral veins spanned by the sector was measured as described by Scanlon and Freeling (1997), and compared to similar sized and staged leaves in which internode sectors were present. These data were used to extrapolate the sector position in the shoot apex, as described in Materials and Methods.

**The developmental timepoint of sector initiation was determined as in Poethig and Szymkowiak (1995) and as described in the text.
Table 4. Wild type meristematic margin sectors

<table>
<thead>
<tr>
<th>Sector (leaf)*</th>
<th>Leaf length</th>
<th>Lateral veins‡</th>
<th>Leaf stage§</th>
<th>Leaf phenotype</th>
<th>Internode girth</th>
<th>Sector position in internode¶</th>
<th>Tissue layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (L3)</td>
<td>55.5 cm</td>
<td>19</td>
<td>J</td>
<td>wild type</td>
<td>8 cm</td>
<td>margin right 2.5 mm-3 mm</td>
<td>L2</td>
</tr>
<tr>
<td>8 (L4)</td>
<td>58 cm</td>
<td>20</td>
<td>J-M</td>
<td>wild type</td>
<td>8 cm</td>
<td>midrib left 2 mm-5 mm</td>
<td>L2</td>
</tr>
<tr>
<td>15 (L8)</td>
<td>77 cm</td>
<td>22</td>
<td>M</td>
<td>wild type</td>
<td>7.4 cm</td>
<td>margin right 1 mm-1.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>15 (L9)</td>
<td>75 cm</td>
<td>21</td>
<td>M</td>
<td>wild type</td>
<td>6.7 cm</td>
<td>midrib left 2 mm-2.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>15 (L10)</td>
<td>74 cm</td>
<td>21</td>
<td>M</td>
<td>wild type</td>
<td>5.9 cm</td>
<td>margin right 1 mm-2 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>15 (L11)</td>
<td>75 cm</td>
<td>19</td>
<td>M</td>
<td>wild type</td>
<td>4.2 cm</td>
<td>midrib left 0.5 mm-1 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>15 (L12)</td>
<td>69 cm</td>
<td>18</td>
<td>M</td>
<td>wild type</td>
<td>3.5 cm</td>
<td>margin right 1.5 mm-2 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>15 (L13)</td>
<td>60 cm</td>
<td>15</td>
<td>A</td>
<td>wild type</td>
<td>2.7 cm</td>
<td>on midrib 0.5 mm wide</td>
<td>L1-L2</td>
</tr>
<tr>
<td>19 (L11)</td>
<td>54 cm</td>
<td>15</td>
<td>A</td>
<td>wild type</td>
<td>3 cm</td>
<td>margin right sector not seen in internode; began in blade</td>
<td>L1-L2</td>
</tr>
<tr>
<td>19 (L12)</td>
<td>49.5 cm</td>
<td>12</td>
<td>A</td>
<td>wild type</td>
<td>2.8 cm</td>
<td>midrib left 2 mm-2.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>19 (L14)</td>
<td>39 cm</td>
<td>12</td>
<td>A</td>
<td>wild type</td>
<td>2.0 cm</td>
<td>margin right 0.5 mm-0.6 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>22 (L7)</td>
<td>51 cm</td>
<td>22</td>
<td>M</td>
<td>wild type</td>
<td>5.9 cm</td>
<td>margin right 1.5 mm-3 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>22 (L8)</td>
<td>66 cm</td>
<td>24</td>
<td>M</td>
<td>wild type</td>
<td>6.2 cm</td>
<td>midrib left 2 mm-4 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>26 (L11)</td>
<td>64 cm</td>
<td>16</td>
<td>M-A</td>
<td>wild type</td>
<td>3.9 cm</td>
<td>margin left 9 mm-12 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>26 (L12)</td>
<td>64 cm</td>
<td>15</td>
<td>A</td>
<td>wild type</td>
<td>2.9 cm</td>
<td>midrib right 8 mm-9 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>26 (L13)</td>
<td>56 cm</td>
<td>14</td>
<td>A</td>
<td>wild type</td>
<td>2.3 cm</td>
<td>margin left 5 mm-5.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>26 (L14)</td>
<td>42 cm</td>
<td>9</td>
<td>A</td>
<td>wild type</td>
<td>2.6 cm</td>
<td>midrib right 6 mm-8 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>27 (6)</td>
<td>60 cm</td>
<td>14</td>
<td>J-M</td>
<td>wild type</td>
<td>7.1 cm</td>
<td>on midrib 2 mm wide</td>
<td>L2</td>
</tr>
<tr>
<td>27 (L7)</td>
<td>65 cm</td>
<td>17</td>
<td>M</td>
<td>wild type</td>
<td>7.0 cm</td>
<td>margin left 0.5 mm-2.5 mm</td>
<td>L2</td>
</tr>
<tr>
<td>27 (L8)</td>
<td>68 cm</td>
<td>20</td>
<td>M</td>
<td>wild type</td>
<td>6.8 cm</td>
<td>midrib right 1 mm-2 mm</td>
<td>L2</td>
</tr>
<tr>
<td>31 (L10)</td>
<td>72 cm</td>
<td>16</td>
<td>M-A</td>
<td>wild type</td>
<td>4.2 cm</td>
<td>margin left 1 mm-1.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>31 (L11)</td>
<td>69 cm</td>
<td>16</td>
<td>A</td>
<td>wild type</td>
<td>3.4 cm</td>
<td>midrib right 1 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>31 (L12)</td>
<td>64 cm</td>
<td>15</td>
<td>A</td>
<td>wild type</td>
<td>2.7 cm</td>
<td>margin left 1 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>32 (7)</td>
<td>54 cm</td>
<td>17</td>
<td>J-M</td>
<td>wild type</td>
<td>4.3 cm</td>
<td>margin left 0.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>32 (8)</td>
<td>63 cm</td>
<td>18</td>
<td>M</td>
<td>wild type</td>
<td>4.5 cm</td>
<td>on midrib 1 mm wide</td>
<td>L1-L2</td>
</tr>
<tr>
<td>32 (9)</td>
<td>68 cm</td>
<td>18</td>
<td>M</td>
<td>wild type</td>
<td>4.1 cm</td>
<td>margin left 0.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>32 (10)</td>
<td>67 cm</td>
<td>18</td>
<td>M</td>
<td>wild type</td>
<td>3.9 cm</td>
<td>midrib right 0.5 mm-1 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>35 (L9)</td>
<td>80 cm</td>
<td>23</td>
<td>M</td>
<td>wild type</td>
<td>6.8 cm</td>
<td>midrib right 7.5 mm-8.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>35 (L10)</td>
<td>79 cm</td>
<td>21</td>
<td>M</td>
<td>wild type</td>
<td>5.9 cm</td>
<td>margin left 5 mm-6 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>43 (L9)</td>
<td>63 cm</td>
<td>18</td>
<td>M-A</td>
<td>wild type</td>
<td>3.3 cm</td>
<td>midrib right 1.5 mm-2.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>43 (L10)</td>
<td>61 cm</td>
<td>14</td>
<td>A</td>
<td>wild type</td>
<td>2.7 cm</td>
<td>margin left 1 mm-1.5 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>43 (L11)</td>
<td>56 cm</td>
<td>13</td>
<td>A</td>
<td>wild type</td>
<td>2.2 cm</td>
<td>midrib right 1 mm</td>
<td>L1-L2</td>
</tr>
<tr>
<td>48 (10)</td>
<td>89 cm</td>
<td>21</td>
<td>M</td>
<td>wild type</td>
<td>6.8 cm</td>
<td>midrib left 1.5 mm</td>
<td>L2</td>
</tr>
<tr>
<td>48 (11)</td>
<td>94 cm</td>
<td>24</td>
<td>M</td>
<td>wild type</td>
<td>6.8 cm</td>
<td>margin right 0.5 mm-2 mm</td>
<td>L2</td>
</tr>
</tbody>
</table>

*Leaf numbers were counted at plant maturity; the leaf closest to the base was designated Leaf 1 (L1).
‡The number of lateral veins per half-leaf with respect to the midvein.
§Basal leaves represented as juvenile (J); middle phase leaves represented as middle (M); apical leaves represented as adult (A).
¶Sector position in the internode measured as described in Materials and Methods.

In Fig. 7, subdivided into old (Fig. 7A) mid-staged (Fig. 7B) and young phytomer sectors (Fig. 7C).

**NARROW SHEATH1 function is localized to meristematic foci**

Clonal sectors of ns mutant tissue induced during different stages of vegetative leaf development reveal that NARROW SHEATH1 function maps to two focal points located in the L2 layer of the SAM (Fig. 7A-C). The boundaries of all mutant sectors, except one sectored leaf designated 20(8) (Fig. 7; Table 1), converge and overlap at two small foci located at the beginnings of the putative, narrow sheath marginal domain. Because a second leaf marked by sector 20 (located two leaves above the non-concordant leaf sector 20(8)), maps to a putative NARROW SHEATH focal point far from the estimated position of sector 20(8), the lone aberrant sector probably represents an error in measurement.

The presence of the non-mutant ns1 gene in L1 meristematic tissue is insufficient for NS1 protein function, as evidenced by the identification of three ns mutant 1/2 leaf sectors that were confined to L2-derived tissues (Table 1; Fig. 6B). No ns non-mutant, meristematic sectors spanned the NS1 foci unless they were partial-L2 layer sectors (Fig. 7B,C). These data indicate...
that NS1 function is non-cell autonomous in the transverse dimension, and is capable of signalling from adaxial L2 meristematic tissue to abaxial tissue, and vice versa.

NS1 function is not required for development of the central leaf domain (Figs 5F, 7), and the ns 1/2 leaf phenotype confirms that the right and left ns margin domains are patterned independently from separate focal points (Fig. 5A-E). These two findings corroborate previous results reported from analyses of dual-aneuploid, ns mosaic plants (Scanlon et al., 2000). No sectors in this particular study were wide enough to include both ns focal points (Fig. 7), such that no chimeric plants displayed the ns mutant leaf phenotype on both leaf margins (Table 1; Fig. 5A-E).

NARROW SHEATH1 function is domain specific and non-cell autonomous within the narrow sheath domain

Meristematic, ns1 mutant sectors spanning the NS focal point conditioned the marginless phenotype, regardless of the overall lateral expanse of the sector. That is, even in the three documented cases where the ns mutant sector is quite narrow (represented as small pink squares in Fig. 7), the presence of non-mutant tissue adjacent to the focal point cannot attenuate the ns mutant phenotype. Therefore, NS1 function cannot move non-cell autonomously from the midrib side of the NS focal point into the focal point. However, loss of NS1 function within the ns domain (shown in yellow in Fig. 7), but restricted to regions marginal to the focal point, has no phenotypic consequence (Figs 5I-L, 7). The data indicate that as long as NS1 function is intact at the focal point, development of the entire ns marginal domain ensues. Thus NS1 is required to initiate the ns domain recruitment signal, but is not required to propagate the signal downstream of the focal point.

NS1 functions during the meristematic stage of leaf development

Among the eight, single-leaf sectors determined to be induced in primordial leaves, at least two comprised all L2-derived tissues and spanned leaf lateral domains estimated to correspond to the NS focal point (Fig. 7A,B; Table 3). None of these single-leaf, marginal sectors, however, conditioned the narrow sheath phenotype (including Fig. 3H). Unfortunately, none of the seven founder-cell-staged sectors identified in this analysis were found to overlap the NS1 meristematic foci (Fig. 7; Table 3). Therefore, a model wherein NS1 functions during the founder-cell stage of development could not be tested. However, these data do indicate that NS1 does not function after the founder cell stage of development (Fig. 5H-I). Instead, the sector data suggest a role for NS1 function in the SAM, during pre-primordial stages of maize leaf development.

The foci of NARROW SHEATH1 function recede laterally as the meristem grows

Comparisons of the estimated meristematic position of the NARROW SHEATH1 foci in older (Fig. 7A) versus younger phytomers (Fig. 7B,C) reveal that NS1 function recedes toward the midrib flank during developmental time. As the plant ages, the NS1 foci progressively move away from the marginal flank of the SAM. This phenomenon probably reflects the dynamics of maize phytomer development. The maize SAM enlarges with every plastochron (Bassiri et al., 1992), whereas the upper (i.e. newer) leaves are smaller than older leaves and may be formed from a smaller number of founder cells. Thus, since in upper leaves the SAM is larger and the founder-cells are fewer, the margin domains of the leaf founder-cells overlap the SAM to a lesser extent (Scanlon and Freeling, 1997). Evidence for the progressive ‘unwinding’ of maize leaf founder-cells from around the growing SAM is provided by the ns 1/2 leaf sectors in this investigation. Mutant sectors in adult leaves usually passed through only one sheath margin, whereas both sheath margins were sectored in older leaves formed from a smaller SAM. These results suggest that the recruitment of founder cells to make up the NS margin domain initiates from different, lateral meristematic regions during maize phytomer development (Fig. 7).

The author thanks Jay Markam, Athens Regional Hospital, for irradiation of corn seed. Thanks to C. McKnight for help in harvesting and photo-documentation of sectored plants.

REFERENCES


Clonal analysis of NARROW SHEATH1


