The SCARFACE gene is required for cotyledon and leaf vein patterning

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SUMMARY

Mechanisms controlling vein patterning are poorly understood. We describe a recessive Arabidopsis mutant, scarface (sfc), which maps to chromosome 5. sfc mutants have vein pattern defects in cotyledons, leaves, sepals and petals. In contrast to the wild type, in which these organs all have linear veins that are continuous with at least one other vein, in sfc mutants these organs' secondary and tertiary veins are largely replaced by small segments of discontinuous veins, which we call vascular islands. Patterning defects are manifest in cotyledon provascular tissue, suggesting that the patterning defect occurs early in organogenesis. sfc mutants have exaggerated responses to exogenous auxin. Analysis of monopteros (mp) sfc-I double mutants suggested that SFC has partially overlapping functions with MP in patterning of both primary and secondary veins.

Key words: Arabidopsis thaliana, Vein patterning, Auxin, SCARFACE, MONOPTEROS

INTRODUCTION

In vascular plants, veins form a network that interconnects all parts of the organism. Veins contain two tissues, xylem and phloem, that transport water and dissolved mineral nutrients (xylem), or sugars, amino acids and other compounds (phloem). Broad organs, such as leaves or cotyledons, have acute physiological needs for veins to be distributed throughout the lamina. Large amounts of water are lost due to transpiration, and this water must be replenished through the xylem. In addition, leaves are the primary site of photosynthesis, and so produce large amounts of sugars. Much of this sugar must be exported from the leaf and transferred to either storage organs or rapidly growing parts of the plant; this transport uses phloem.

Strategies used to achieve a distributed pattern of veins in leaves vary between different groups of plants. For example, monocots typically show parallel venation, in which major veins extend parallel along the proximal/distal leaf axis and largely lack major vein branching. In contrast, dicot leaves typically contain branched veins, and groups of dicots vary with respect to numbers and positions of vein branches. Although leaf vein patterns have been well characterized, almost nothing is known about the developmental mechanisms that specify these patterns.

One molecule that has been proposed to play a role in vein patterning is the plant hormone auxin. Auxin is synthesized in apical parts of the plant, and is moved basipetally by a polar transport mechanism (reviewed by Jones, 1998). It has been proposed that early in organogenesis, polar auxin transport streams are established that later specify positions for vein development (Sachs, 1991). Recently, several studies have investigated possible roles of polar auxin transport in leaf development through the use of auxin polar transport inhibitors (Mattsson et al., 1999, Sieburth, 1999; Tsiantis et al., 1999). In all three studies, seedling growth in the presence of polar auxin transport inhibitors resulted in multiple morphological changes, including altered leaf vein patterns. However, we do not know whether these changes were the direct result of reduced transport or an indirect result of increased auxin concentrations, and we also do not yet know which molecules effected the changes in vein pattern in response to the altered auxin conditions.

To investigate vein patterning, we have conducted a screen for Arabidopsis vein patterning mutants, and in this study, we present our characterization of plants containing mutations at the scarface (sfc) locus. sfc mutants produce organs with defects in the axial continuity of secondary and higher-order veins. Our investigations suggest that sfc mutants have heightened auxin responses, and that SFC functions in processes closely related to those of MONOPTEROS.

MATERIALS AND METHODS

Mutagenesis and mutant screening

We mutagenized seeds of Arabidopsis thaliana ecotype Landsberg erecta (Ler) for 3 hours in 130 mM solutions of EMS (ethane methyl sulfonate; Sigma). The self-fertilized progeny of these seeds were collected as 5259 individual M2 families, and screened for morphological abnormalities after 10 days growth on germination.
medium (GM; 0.5× Murashige and Skoog basal salts (Sigma), 1% sucrose, 0.5 g/l MES buffer [2-[N-Morpholino]ethanesulfonic acid; Sigma], 0.7% phytagar (GIBCO-BRL), pH 5.7). Abnormal seedlings were fixed in a solution of 3:1 ethanol:glacial acetic acid, cleared in saturated chloral hydrate (Sigma), and vein patterns were examined microscopically using dark-field illumination. After identifying lines that segregated putative mutants, we backcrossed heterozygotes to Ler plants through four successive cycles before proceeding with detailed phenotypic analyses. Independent lines with mutant phenotypes similar to sfc-1 were crossed to sfc-1 and examined in the F1 generation to assess complementation.

**Growth conditions**

For detailed physiological and anatomical analyses, seeds were surface-sterilized and plated on GM in Petri plates or magenta boxes. They were cold-stratified at 4°C for 3 days, and then transferred to a TC-30 Conviron controlled environment chambers at 24°C, under constant light at 100-120 μmol/m²/second. Seedling age refers to days after transfer from the cold stratification.

**Genetic mapping**

We extracted the DNA of more than 100 individual F2 mutant seedlings from crosses of sfc-1 heterozygotes to ecotype Columbia (Dellaporta et al., 1983). This DNA was genotyped at several microsatellite loci that are polymorphic between ecotypes Ler and Columbia (Col-0) (Bell and Eck er 1994), allowing us to narrow the map position down to chromosome 5. For more than 100 sfc-1 mutant plants, we combined pairwise recombination frequencies between the microsatellite markers nga225, nga249, and nga106 and the sfc-1 locus (using MAPMAKER software (Whitehead Institute, version 2.0) to place the SFC gene approximately 7 cM south of nga249.

**Root elongation assays**

Root elongation assays were based on the method of Estelle and Somerville (1987). We grew Ler and segregating populations of sfc-1 on vertically oriented GM plates for 4 days, then transferred the seedlings to fresh GM or GM supplemented with either indole-3-acetic acid (Sigma), or 2,4-dichlorophenoxyacetic acid (Sigma), (10⁻⁶ M to 10⁻¹⁰ M in a 10-fold dilution series). After transfer, the position of each root tip was marked on the plate, and then marked again after 3 days of additional growth. Each root's increase in length was then determined by measuring digitized images of the roots with NIH Image 1.61 software. We calculated the mean increase in root length at each concentration of hormone and divided this by the mean change in root length on hormone-free medium to calculate the percentage elongation for each genotype at each hormone concentration.

**Histology**

Leaves and cotyledons were fixed (2.5% gluteraldehyde, 3% paraformaldehyde, 0.1 M sodium phosphate, pH 7.3) by first vacuum infiltrating for 5 minutes, followed by microwave fixation (Pelco model 3420). Following fixation, the tissue was rinsed (0.1 M NaPO₄ buffer, pH 7.3), and a secondary fix performed using 1% osmium tetroxide (in 0.1 M NaPO₄ buffer, pH 7.3). Following a buffer rinse, the tissue was dehydrated in an ethanol series (10%, 30%, 50%, 70%, 95%, 100%), and infiltrated with Spurr resin (steps of 25%, 50%, 75%, and 100%). 1 μm sections were prepared and stained with 1% toluidine blue (w/v) in 1% sodium borate (w/v), and photographed using an Olympus BX-50 microscope.

To examine embryonic cotyledon vein patterning, embryos were dissected from dry seed that had imbibed in 70% ethanol overnight. They were transferred to saturated chloral hydrate for 24 hours, and examined as whole-mounts using DIC optics.

**Double mutant analyses**

For double mutant analysis with auxin-resistant mutants, we crossed sfc-1 heterozygotes to plants that were homozygous for one of each of the following mutations: aux1-7 (Pickett et al., 1990), axr1-3 (Lincoln et al., 1990), axr2-1 (Timp te et al., 1992), and axr4~2 (Hobbie and Estelle, 1995). From crosses of sfc-1 to aux1-7 and axr1-3, we identified F3 families in which all individuals (except the sfc-1 homozygotes) were resistant to 2×10⁻⁷ M 2,4-D. All seedlings within these families were presumed to be homozygous for the specific auxin-resistant mutation, and sfc-1 mutants segregated within these families at near 3:1 ratios (χ² P=0.41, 0.46 for double mutants of sfc-1 with axr1-3, and aux1-7 respectively). Because no F3 families that were homozygous for axr4~2 were identified, seedlings from these families were scored separately for the segregation of sfc-1 and axr4~2. sfc-1 segregated within F3 families of the dominant axr2-1 mutation at near-expected ratios (χ² P=0.07).

To obtain double mutants of sfc-1 and the seedling lethal mutants mpT370 (Berleth and Jürgens, 1993) and rty-5 (Windsor and Waddell, pers. comm.), heterozygotes were crossed. Double mutants were identified within F2 families that segregated for each single mutant, and were easily distinguished by the presence of the distinct morphological features characteristic of each parental mutant phenotypes. For each of the double mutant combinations, we examined at least 191 seedlings from two or more F2 families. The segregation ratios were subjected to χ² analysis, with resulting P values of 0.2, and 0.003 for expected dihybrid segregation ratios of sfc-1 with mpT370 and rty-5.

**RESULTS**

To identify genes required for normal vein patterning in leaves and cotyledons, we screened for vein patterning mutants among the progeny of 5259 EMS-mutagenized Arabidopsis plants. We identified several alleles of one mutant with vein pattern defects in both the cotyledons and leaves. In contrast to the wild type, which has continuous veins in cotyledons and leaves (depicted in Fig. 1, shown in Fig. 2A,C), in these mutants some vein classes in both leaves and cotyledons were replaced by isolated clusters of vascular tissue (Fig. 2B,D). We called these putative mutants *scarface* (*sfc*) in reference to this disfigured vasculature.

We observed segregation of *sfc* mutants in a ratio consistent with a single recessive lesion (3.1:1; n=148). Using molecular markers (SSLPs; simple sequence length polymorphisms), we mapped the *SFC* locus to chromosome 5, between markers nga106 and nga249 (Bell and Eck er, 1994). In total, we

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**Fig. 1.** Cotyledon and leaf vein patterns in wild-type plants. (A) Cotyledon vein pattern. (B) Leaf vein pattern. Both cotyledons and leaves contain primary veins (1') and secondary veins (2'). Leaves also contain tertiary (3') veins and an intramarginal vein (IV). The gray box in B indicates the region from which the paradermal sections shown in Fig. 3 were obtained; dashed lines indicate positions used for transverse sections shown in Fig. 3.
We compared cotyledon vein patterns of 14-day old seedlings. Observations indicate that approximately 5% of the VIs appeared in positions atypical for veins. These short vein-like segments, which we refer to as vascular islands (VIs), contained a central primary vein that was continuous with other veins (Fig. 3C-E). These observations indicate that SFC function is required for the formation of a normal continuous pattern of cotyledon secondary veins.

Vascular defects in sfc-1 mutants

Cotyledon vein patterns

We compared cotyledon vein patterns of 14-day sfc-1 and wild-type seedlings. In sfc mutants, cotyledons resembled the wild type in gross morphology, except that they were smaller, slightly epinastic, and often had anthocyanin pigment accumulation on their abaxial side. Vein patterns in wild-type cotyledons feature a primary vein that is continuous with the hypocotyl vasculature and extends the length of the proximal/distal axis, and typically four secondary veins that branch from the primary vein (shown in Fig. 2A, depicted in Fig. 1A). As in the wild type, sfc cotyledons invariably contained a central primary vein that was continuous with the hypocotyl vasculature and that extended the length of the cotyledon proximal/distal axis (Fig. 2B). However, in contrast to the wild type, the secondary veins were mostly replaced by short vein-like segments, which we refer to as vascular islands (VIs). Most of the VIs appeared in positions where secondary veins might be found in a wild-type cotyledon, however, approximately 5% of the VIs appeared in positions atypical for veins (e.g., very close to the cotyledon margin). These observations indicate that SFC function is required for the formation of a normal continuous pattern of cotyledon secondary veins.

Leaf vein patterns

We also compared wild-type and sfc-1 leaf vein patterns. Arabidopsis wild-type leaf vein pattern, shown in Fig. 2C and depicted in Fig. 1B, has been described previously (Kinsman and Pyke, 1998; Candela et al., 1999); a central primary vein connects with the vascular tissue of the stem, and extends the length of the leaf proximal/distal axis. Between 8 and 12 secondary veins branch from the midvein, and tertiary veins branch from the secondary veins.

The leaves produced by sfc mutants resembled those of the wild type in gross morphology, adaxial/abaxial epidermal patterning, and color; however, they were typically only half the size of the wild type. The vein pattern of the first leaf of a sfc-1 mutant is shown in Fig. 2D. The leaf primary vein and the circuit of intramarginal veins (depicted in Fig. 1B) were typically contiguous in sfc mutants, but most of the rest of the leaf lamina contained VIs of varying lengths. All rosette and cauline leaves produced by sfc-1 plants showed similar vein patterns (data not shown). These results indicate that SFC function is required for the formation of the normal pattern of continuous veins in both leaves and cotyledons.

Vein patterns of other organs

To determine whether the sfc mutation affected vein continuity in other organs, we compared veins of roots, hypocotyls, inflorescence stems, sepals, and petals for sfc-1 and wild type. In wild type, sepals contained 3 to 5 veins that extended along the proximal/distal axis (Fig. 2E). In sfc-1 mutants, sepals contained a reduced number of intact veins, and some VIs (Fig. 2F). Wild-type petals contained a central primary vein that extended most of the length of the organ, and three to five secondary veins (occasionally bifurcated) that branched from the primary vein (Fig. 2G). Petals of sfc mutants contained an intact primary vein, but within the petal lamina, secondary veins were replaced by a small number of VIs (Fig. 2H). For sfc roots, hypocotyls and inflorescence stems, the veins resembled those of the wild type in both the radial and longitudinal axes (data not shown). Taken together, these observations suggest that sfc-1 vascular defects are limited to the secondary and higher order veins of flat organs (cotyledons, leaves, sepals, petals).

Anatomical characterizations

To characterize the anatomy of sfc-1 mutants, we compared sections prepared from resin-embedded leaves and cotyledons. Our previous characterizations used dark-field illumination of cleared intact tissue, which allowed us to observe xylem tracheary elements. To determine whether other vascular cell types (e.g., phloem sieve tube elements) formed connections between VIs, we compared paradermal sections (parallel to leaf surface) of leaf tissue prepared from the middle of the leaf adjacent to the primary vein (region depicted by the gray box in Fig. 1B). Sections from wild type are shown in Fig. 3A,B; veins were all continuous with other veins on at least one end. Sections from sfc-1 are shown in Fig. 3C-E. Some veins matched those observed in the wild type, and were continuous with other veins on at least one end (data not shown). Other veins were composed of irregularly shaped vascular cells and corresponded to VIs. When we compared the serial sections of the VI regions, there were no vascular cell types that were continuous with other veins (Fig. 3C-E). These observations confirmed that the VIs were discontinuous.
To determine whether sfc defects were restricted to vascular tissue, we compared transverse sections of leaves, taken at their midpoint (region of section depicted in Fig. 1B). In the wild type, leaf mesophyll cells are differentiated into palisade parenchyma on the adaxial (upper) side and spongy parenchyma on the abaxial (lower) side, and the primary vein is surrounded by a ring of bundle sheath cells (Fig. 3F). These pattern elements were also present in the sfc mutant (Fig. 3G).

Cotyledon transverse sections were also compared; pattern elements of the two genotypes were similar (data not shown). These data suggest that sfc patterning defects in these organs are limited to the vascular tissue.

To determine whether the sfc vascular defects extended to the abaxial/adaxial patterning of vascular cell types, we compared transverse sections of vascular bundles of wild type and sfc mutants. Wild-type and sfc leaf primary veins were shown in Fig. 3H,I, and cotyledon veins in Fig. 3J,K. In both genotypes, vascular bundles were organized in the normal pattern, with xylem tissues toward the adaxial surface and phloem tissues oriented toward the abaxial surface, although in sfc mutants, the adaxial/abaxial alignment was occasionally rotated by approximately 20°.

To determine whether the abaxial/adaxial vascular cell type patterning was intact in VIs, we prepared transverse sections from cotyledons at a position 30% the distance from the lamina base to the distal end (position depicted in Fig. 1A). In the wild type, normal secondary veins were typically present in these positions, while in sfc mutants, this region invariably contained VIs. Radial organization of wild-type secondary veins resembled the primary vein (compare Fig. 3J to L). Two different VI cross sections are shown (Fig. 3M,N). The adaxial/abaxial orientations of xylem and phloem were preserved in the sfc VIs. Taken together, these anatomical analyses indicated that the sfc mutation disrupts normal axial (longitudinal) patterning of secondary and higher-order veins.

**Provascular patterning is disrupted in sfc mutants**

The sfc vein pattern defect could, in theory, result from either an initial patterning defect, or from a normal pattern that failed to differentiate uniformly. To distinguish between these possibilities, we compared provascular patterning in sfc-1 mutants and the wild type. Provascular tissue is the first morphological indication of vein differentiation, and is distinguishable because the cells are elongated. In Arabidopsis, cotyledon vein pattern is established during embryogenesis, and can be detected in cotyledons of dry seed embryos in the same pattern as the veins of fully differentiated cotyledons (Sieburth, 1999). Therefore, to assess sfc provascular
patterning, we compared cotyledons of dry seed embryos obtained from a plant that segregated for sfc-1 mutants to dry seed embryos of wild-type plants.

In the wild-type dry seed embryos, the cotyledon provascular tissue pattern matched the mature cotyledon vein positions (Fig. 4A,B). However, among embryos from heterozygous sfc-1 parents, approximately one quarter had cotyledons with normal primary vein provascular tissue, but in secondary vein positions, the provascular tissue was interrupted and often also disorganized (Fig. 4C,D). These observations suggest that sfc vein axial discontinuities resulted from defects in provascular patterning.

sfc mutants have altered auxin responses

Because aspects of vascular cell type differentiation and vascular patterning have been linked to the hormone auxin (reviewed by Aloni, 1995), it is possible that the sfc phenotype resulted from defects in auxin perception. To test whether auxin perception was altered in sfc-1, we compared the efficacy of auxin to inhibit root elongation for sfc and wild-type seedlings (Fig. 5). In the wild type, exogenous auxin inhibited root elongation and the ID$_{50}$ (dose conferring 50% inhibition of root elongation; Maher and Martindale, 1980) for IAA was 2.7×10$^{-8}$ mol/l. In sfc-1 mutants, exogenous auxin also inhibited root elongation (Fig. 5). However, in contrast to the wild type, for sfc-1 mutants, the ID$_{50}$ was 5.8×10$^{-9}$ mol/l, about a 20-fold increase in sensitivity. We obtained similar root elongation responses in replicates and in experiments using the synthetic auxin 2,4-D in the place of IAA. These results indicate that, at least in roots, sfc-1 mutants showed increased auxin sensitivity.

We next wanted to determine whether SFC function is related to that of the auxin-response factor gene, MONOPTEROS (MP). Molecular characterization of MP has shown that it encodes a transcription factor that binds to auxin response elements in the promoters of auxin-regulated genes (Hardtke and Berleth, 1998) and phenotypic characterizations have shown that mp mutants have no root, a highly reduced hypocotyl, and cotyledons with greatly reduced vein patterns (Berleth and Jürgens, 1993). We constructed double mutants using mp$^{T370}$. This is a strong allele, and cotyledons contained a primary vein that typically extended only half the length of the cotyledon and no secondary veins (Berleth and Jürgens, 1993) (Figs 6, 7A). We used DIC optics to assess the presence of provascular tissue in mp$^{T370}$ cotyledons. We did not observe provascular tissue in the lamina where secondary veins typically appear, however for mp$^{T370}$ primary veins, we invariably observed provascular tissue that extended from the distal end of the mp primary vein (averaging close to 50% the length of the cotyledon) into the distal-most 2/3 of the organ (n=54/54, Fig. 7G,H). These observations agree with, and extend those of Przemeck et al. (1996), and suggest that mp$^{T370}$ vascular defects include both vein patterning and differentiation of vascular cell types from provascular tissue.

We identified the sfc-1 mp$^{T370}$ double mutants among the seedlings that were missing basal seedling parts, like the mp single mutant. The sfc-1 mp$^{T370}$ double mutant had cotyledons that contained a short primary vein, no secondary veins and a variable number of small VIs (Fig. 7D). The differentiated primary vein of the double mutant tended to be shorter than that of the mp single mutant (Fig. 6). More strikingly, in the double mutant the primary vein provascular tissue extended distally no more than 2 elongated cells beyond the differentiated primary vein (n=39/39, Fig. 7I). This loss of
primary vein provascular tissue was not observed in either single mutant. In addition, sfc-1 mp T370 double mutants also had a unique VI phenotype in which small VIIs were scattered in an apparently random pattern in the cotyledon lamina. The observation of a unique and enhanced phenotype (the loss of primary vein provascular tissue and the complete randomization of VI positions) in the sfc-1 mp T370 double mutant suggests that MP and SFC have partially overlapping roles in patterning of cotyledon primary and secondary veins.

We also characterized double mutants between sfc-1 and the auxin-resistant mutants axr1-3, axr2, axr4-2 and aux1-7 (Estelle and Somerville, 1987; Hobbie and Estelle, 1995; Maher and Martindale, 1980; Pickett et al., 1990; Wilson et al., 1990). We did not observe significant changes in cotyledon vein pattern for any of these double mutants (data not shown). These results suggest that the sfc vein pattern defects were not affected by the reduced auxin responses resulting from auxin-resistant mutations. We also analyzed double mutants between sfc-1 and the auxin-overaccumulating mutant rty-5 (Boerjan et al., 1995; King et al., 1995; Windsor and Waddell, personal communication). The vein pattern of these double mutants also matched that of the sfc single mutant (data not shown), suggesting that interrupted sfc secondary veins could not be ameliorated simply by increasing auxin levels.

sfc-1 seedling phenotype

To determine what other phenotypes accompanied the interrupted vein pattern of sfc mutants, we characterized the sfc-1 seedling phenotype. sfc-1 mutants could first be distinguished from the wild type after 3 days by their smaller size. At 14 days, the wild-type cotyledons and first leaf pair were fully expanded; sfc mutants at this stage had organs that were approximately 50% the size of the wild type (Fig. 8A,B).

The morphology of sfc-1 mutants matched wild-type plants...
the mutant plants infertile. Taken together, these observations
suggest that SFC function is required for normal activity of
both axillary meristems and the shoot apical meristem.

**DISCUSSION**

We have described the isolation and characterization of
mutants at the SCARFACE (SFC) locus. sfc mutants have
cotyledons, leaves, sepals and petals with discontinuous
secondary and higher order veins. That all four of these organ
types show similar defects indicates that a common mechanism
is used in secondary vein patterning in these organ types.

**Multiple genes function to specify continuity of
cotyledon secondary veins**

In addition to SFC, mutations at four other loci (AXR6, CVP1,
CVP2 and MP) also define genes with functions in cotyledon
vein patterning (Hobbie et al., 2000; Carland et al., 1999;
Berleth and Jürgens, 1993). All these mutants show similar
cotyledon vein pattern defects; cotyledon primary veins are
largely intact, and cotyledon secondary veins are disrupted.
That primary veins are intact and secondary veins are
disrupted by mutations at five loci provides strong evidence
for separable pathways specifying patterning of these two
vein types.

Because auxin has been implicated in vein patterning, a
variety of experiments have been carried out to determine
whether auxin responses in these mutants are intact. Plants
mutant for mp show reduced auxin transport, pin-like flowers,
and the loss of structures (root, hypocotyl) associated with
auxin signaling (Przemek et al., 1996). In contrast, cvp1 and
cvp2 mutants have normal auxin transport, normal flower
structures, and no morphological defects (Carland et al., 1999).
Auxin transport has not been analyzed in plants homozygous
mutant at the axr6 or sfc loci, however their vascular
phenotypes do not match the pattern observed for wild-type
plants treated with auxin polar transport inhibitors (Sieburth,
1999; Mattsson et al., 1999) or the pattern of plants mutant for
genes proposed to encode components of the polar auxin
transport machinery (Okada et al., 1991; Mattsson et al., 1999).

Auxin perception has been measured for axr6 and sfc mutants
using root elongation assays; whereas axr6 mutants show
reduced auxin response (Hobbie et al., 2000), sfc mutants show
enhanced auxin response. Thus, a clear picture of the
relationship between auxin, the products of these five genes,
and secondary vein patterning has yet to emerge.

**The sfc mp double mutant phenotype suggests
overlapping roles**

One approach to sorting out whether the genes identified by
this collection of mutants with discontinuous cotyledon veins
function in related processes is to analyze double mutants. We
have begun this analysis by characterizing sfc mp double
mutants. One element of the unique double mutant phenotype
was the sharp reduction in cotyledon primary vein provascular
structure. In the mp single mutant, we observed primary vein
provascular tissue that extended into the distal quarter of
cotyledons, but when mp was combined with sfc, this
provascular tissue was essentially eliminated. These
observations suggest that SFC and MP have partially redundant
roles in cotyledon primary vein patterning. However, that some
primary vein still remains in the double mutant indicates that other genes also contribute to this process.

Another element of the unique $sfc$ $mp$ double mutant phenotype was the small VIs that were apparently randomly distributed. In $sfc$ single mutants, secondary veins are replaced by large VIs that mostly appear in positions similar to those of secondary veins. The reduced size of VIs in the double mutant suggests that MP contributes to VI formation in $sfc$ single mutants. That VI position does not completely match secondary vein positions in the $scf$ single mutant indicates a defect in secondary vein patterning. Because this patterning defect is so dramatically enhanced in the $scf$ $mp$ double mutant suggests that SFC and MP also have partially redundant roles in cotyledon secondary vein patterning.

**SFC might function as a negative regulator of auxin responses**

Root elongation experiments indicated that $sfc$ mutants showed enhanced sensitivity to exogenous auxin. Because we isolated six recessive mutant alleles with the same mutant phenotype, it is reasonable to expect that these represent loss-of-function alleles. One way to reconcile increased auxin sensitivity in a loss-of-function allele is if SFC functions as a negative regulator. Loss of a putative negative regulator would be expected to allow increased activation of the affected pathway, which in the case of auxin signaling might be detectable as increased sensitivity.

Possible function of SFC as a negative regulator might explain the presence of VIs in $sfc$ $mp$ double mutants. $mp$ mutants generally do not have VIs (Przemeck et al., 1996), however VIs were a regular feature of $sfc$ $mp$ double mutants. Molecular characterization of $MP$ indicates that it encodes a transcription factor that is likely to regulate gene expression in response to auxin (Hardtke and Berleth, 1998). The numerous morphological defects observed in $mp$ mutants, including vein axial defects, are thus explained by the loss of auxin-derived positive signals. Residual positive signals for secondary veins or VIs might be amplified in $sfc$ $mp$ double mutants if $sfc$ mutations result in the loss of negative regulation. This possibility leads to the question of the origin of possible residual positive signals. Because $mp^{T370}$ is believed to be a null allele, VI formation in the $mp^{T370}$ $sfc$-1 double mutant suggests the existence of other positive signals.

Reconciling a role for $SFC$ as a negative regulator with its redundancy with $MP$ is more problematic. $MP$ is proposed to control gene expression in the context of providing positive auxin signaling (Hardtke and Berleth, 1998). The simplest characterization of $SFC$ is to interpret it as a transcription factor that is likely to regulate gene expression in response to auxin (Hardtke and Berleth, 1998). The numerous morphological defects observed in $mp$ mutants, including vein axial defects, are thus explained by the loss of auxin-derived positive signals. Residual positive signals for secondary veins or VIs might be amplified in $sfc$ $mp$ double mutants if $sfc$ mutations result in the loss of negative regulation. This possibility leads to the question of the origin of possible residual positive signals. Because $mp^{T370}$ is believed to be a null allele, VI formation in the $mp^{T370}$ $sfc$-1 double mutant suggests the existence of other positive signals.

**Apical dominance and transport of leaf-generated signals**

Apical dominance has been linked to auxin signaling, although its precise role in this process continues to be elusive (Cline, 1994; Stirnberg et al., 1999). The loss of apical dominance in $sfc$ mutants could be the result of defects in dominance-promoting signals reaching the axillary meristems, or defects within the axillary meristems themselves. Little is known about how axillary meristems become activated, although a role for auxin as a positive signal has been proposed based on increased auxin levels in axillary buds following dominance release (Hillman et al., 1977). If auxin does function positively in axillary meristem activation, then the increased auxin sensitivity of $sfc$ mutants might allow activation regardless of the presence of dominance-promoting signals. However, auxin is also considered a likely candidate for at least part of the signalling pathway that promotes apical dominance, and the loss of apical dominance in mutants is often accompanied by reduced auxin or reduced auxin responses (e.g. see Ruegger et al., 1997). In stems, auxin polar transport occurs in specialized cells that are associated with vascular tissue. The interrupted veins in $sfc$ mutants might also interrupt delivery of auxin or other apical dominance-promoting signals. Vascular tissue has also been proposed to transport leaf-generated signals for floral induction (King and Zeevaart, 1973; Lang et al., 1977). The strong reduction in flowering of $sfc$ mutants might be explained if $sfc$ vascular discontinuities also decreased the delivery of putative leaf-generated floral-induction signals to the shoot apical meristem.

**Models for vein patterning**

Two models have been proposed for patterning of veins (reviewed by Nelson and Dengler, 1997). One model, the canalization of auxin flow hypothesis, suggests that linear paths for veins are first specified by the direction of auxin polar transport (Sachs, 1991). An alternative model, the diffusion-reaction prepattern hypothesis, proposes that initially random signals are refined by the combined action of an autocatalytic activator and a long-distance suppressor (Meinhardt, 1982; Koch and Meinhardt, 1994). The presence of VIs, and the malleability of their positions within cotyledon lamina in different genetic backgrounds, is difficult to explain in terms of the canalization model. Molecular and more extensive genetic characterizations of $SFC$, $CVP1$, $CVP2$ and $AXR6$ should provide the data for distinguishing between these two models, or might suggest new models to explain the developmental events that underlie vein patterning.

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