

Corrigendum

SCD1 is required for cell cytokinesis and polarized cell expansion in *Arabidopsis thaliana*

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An error in the title of this article was not corrected before going to press.

The title should read:

SCD1 is required for cytokinesis and polarized cell expansion in *Arabidopsis thaliana*

The authors apologise to readers for this mistake.

SCD1 is required for cell cytokinesis and polarized cell expansion in *Arabidopsis thaliana*

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SUMMARY

In the leaf epidermis, guard mother cells undergo a stereotyped symmetric division to form the guard cells of stomata. We have identified a temperature-sensitive *Arabidopsis* mutant, *stomatal cytokinesis-defective 1-1* (*scd1-1*), which affects this specialized division. At the non-permissive temperature, 22°C, defective *scd1-1* guard cells are binucleate, and the formation of their ventral cell walls is incomplete. Cytokinesis was also disrupted in other types of epidermal cells such as pavement cells. Further phenotypic analysis of *scd1-1* indicated a role for *SCD1* in seedling growth, root elongation and flower morphogenesis. More severe *scd1* T-DNA insertion alleles (*scd1-2* and *scd1-3*) markedly affect polar cell expansion, most notably in trichomes and root hairs. *SCD1* is a unique gene in

Arabidopsis that encodes a protein related to animal proteins that regulate intracellular protein transport and/or mitogen-activated protein kinase signaling pathways. Consistent with a role for *SCD1* in membrane trafficking, secretory vesicles were found to accumulate in cytokinesis-defective *scd1* cells. In addition the *scd1* mutant phenotype was enhanced by low doses of inhibitors of cell plate consolidation and vesicle secretion. We propose that *SCD1* functions in polarized vesicle trafficking during plant cytokinesis and cell expansion.

Key words: Cell Plate, Cell Expansion, Cytokinesis, DENN domain, Guard cell, Vesicle trafficking, *Arabidopsis thaliana*

INTRODUCTION

Plant morphogenesis is controlled by the rate and orientation of cell division and cell expansion. Cytokinesis is mediated by the assembly of the cell plate, an organelle whose position is governed by developmental cues. Morphological studies have elucidated the dynamic process of cell plate formation. The phragmoplast, a specialized cytoskeletal structure composed of microtubules and microfilaments, forms at the end of anaphase to guide cell plate assembly and expansion. During cytokinesis secretory vesicles carrying membrane and cell wall components are guided along the phragmoplast toward its center, where they fuse to form a membranous tubular-vesicular network (TVN) (Samuels et al., 1995). As additional vesicles are added, the TVN gradually develops into a smoother more plate-like structure that expands outward toward the margin of the cell, until it ultimately fuses with the parental plasma membrane yielding two individual cells separated by a new cell wall.

Several genes involved in plant cytokinesis have been revealed recently through the characterization of mutant plants that either display defects in the determination of the division plane or that show aberrant cell plate formation. Mutants that fall into the first class such as *ton/fass*, *tangled* and *discordia*

I show defects in the proper alignment of the division machinery and thus have misoriented cell walls (Smith, 2001). In contrast, mutants that are defective in the construction of the cell plate have multinucleated cells with incomplete or missing cell walls (Nacry et al., 2000; Söllner et al., 2002).

Several embryo and seedling-defective *Arabidopsis* mutants with multinucleated cells and incomplete cell walls have been described. Cloning of these genes has revealed three classes of proteins essential for cell plate formation. Genes in the first class include *KNOLLE* (Lukowitz et al., 1996) and *KEULE* (Assaad et al., 2001) that are required for cell plate membrane fusion. *KNOLLE* encodes a homolog of syntaxin, a mammalian plasma membrane SNARE (soluble N-ethylmaleimide-sensitive fusion attachment protein receptors) required for the fusion of exocytic vesicles, whereas *KEULE* encodes a homolog of yeast Sec1p that regulates syntaxin function. These factors have been shown to interact both genetically and biochemically, indicating that they are components of the same cell plate membrane fusion pathway (Assaad et al., 2001). Mutations in a second class of genes required for cell plate biogenesis affect the synthesis of the primary cell wall. Cell wall biosynthesis, which is initiated within the cell plate lumen, is thought to stabilize the cell plate as it expands outward toward the parental plasma membrane (Verma, 2001).

KORRIGAN (*KOR*) (Nicol et al., 1998; Zuo et al., 2000) and *CYTI* (Lukowitz et al., 2001) are two genes involved in cell wall biosynthesis that encode an endo-1, 4- β -glucanase and a mannose-1-phosphate guanylyltransferase, respectively. *HINKEL* (Strompen et al., 2002), which encodes a kinesin-related protein, defines a third class of genes required for phragmoplast-mediated expansion and cell plate guidance. Recent reverse genetic studies have linked the function of *HINKEL* and division plane-localized components of a mitogen-activated protein kinase (MAPK) pathway in the control of phragmoplast expansion (Nishihama et al., 2002).

The identification of these genes has yielded insight into the molecular mechanisms that govern plant cytokinesis. However, given the complexity of this process, it is clear that genetic screens for other components of the cytokinetic machinery are not yet saturated. For example, genes involved in polarized trafficking of exocytic and endocytic cell plate vesicles have not been identified to date. Severe loss-of-function mutants in such genes may be difficult to isolate and characterize as they may encode proteins required for both cell division and expansion. Alternatively, functionally redundant genes involved in embryonic cell division may not be revealed in a phenotypic screen for embryo and seedling defective cytokinesis mutants. However, it may be possible to isolate mutants that display tissue- and/or cell-type-specific defects in cell division when the requirement for these genes is under developmental or cell-type-specific control. Recently, several examples of such cytokinesis-defective mutants have been identified including *tsol* (Hauser et al., 2000; Song et al., 2000), which affects floral tissue development, the root morphogenesis mutants *pleiade* and *hyade* (Müller et al., 2002), the pollen cytokinesis-defective mutant *gem1/mor1* (Park and Twell, 2001), and *cyd1*, which displays variable cell-type specific defects in stomata, roots, and other cell types (Yang et al., 1999).

Stomata are found in the leaf epidermis and consist of two guard cells surrounding a pore. They function to regulate gas exchange between the atmosphere and internal air spaces of leaves. Stomatal density is determined by environmental and internal cues. Unlike most other leaf cell types, guard cells are continually generated throughout leaf development by the differentiation and subsequent division of guard mother cells (Nadeau and Sack, 2002a; Nadeau and Sack, 2002b). The stereotyped divisions of guard mother cells that produce two guard cells arranged with mirror-like symmetry facilitates the identification of cytological defects when using microscopy-based mutant screens for genes that participate in cytokinesis. Cytokinesis-defective stomata have been readily observed in plants treated with caffeine (Galatis and Apostolakis, 1991; Terryn et al., 1993), an agent that disrupts the consolidation of the cell plate membrane system (Samuels and Staehelin, 1996). As shown previously by Terryn et al. (Terryn et al., 1993), guard cells from caffeine-treated *Arabidopsis* plants (Fig. 1A-C) resemble the guard cells observed in mutants that are defective in components of the general cytokinetic machinery, including the cell plate-associated mitogen-activated protein kinase (MAPK) kinase kinase, NPK1 (Jin et al., 2002; Nishihama et al., 2001), its associated kinesin-like protein, NACK1 (Nishihama et al., 2002) and the secretory membrane fusion factor, KEULE (Söllner et al., 2002). Similar abnormal stomata are present in *cyd1* (Yang et al., 1999) and several

other recently identified *Arabidopsis* cytokinesis-defective mutants (Söllner et al., 2002) whose genes have not yet been cloned. Because of their sensitivity to defects in cell division, we reasoned that additional genes required for general plant cytokinesis would be revealed through the identification of weaker non-lethal mutations that mainly disrupt stomatal development. Here we report the identification of a conditional mutant allele that severely disrupts the formation of the cell plate during guard mother cell cytokinesis, and the cloning of its associated gene, *SCD1* (stomatal cytokinesis-defective 1). We have analyzed T-DNA::*scd1* insertion alleles and demonstrated that *SCD1* is also critical for polarized cell expansion and most likely functions in regulating exocytic vesicle trafficking.

MATERIALS AND METHODS

Plant growth conditions

For mutagenesis, backcrosses, and control experiments we used *Arabidopsis thaliana glabrous1* (*gl1*) Columbia ecotype (Col) plants. Plants grown on soil (Germination mix Conrad Fafard, Agawam, MA) were illuminated under long days (14 hours light, 10 hours dark) or continuously and grown at 22°C or at 16-18°C. Standard agar growth medium contained 0.5 \times MS salts (Murashige and Skoog, 1962), 1% w/v sucrose and 0.6% w/v Phytagar (MS-Suc) (Gibco-BRL, Rockville, MD). For seedlings grown on vertical plates the concentration of Phytagar was increased to 1.0% or 1.5% w/v. For the inhibitor studies, sterilized MS-Suc 0.8% w/v agar medium was cooled to 60°C before adding inhibitors.

Inhibitor studies

Seeds from segregating wild-type and heterozygous *scd1-1* and *scd1-2* plants were germinated on MS-Suc plates at 22°C under continuous light. After 3-4 days, phenotypically wild-type and mutant seedlings were transplanted and grown vertically for an additional 4-5 days on freshly prepared MS-Suc 0.8% w/v agar plates in the presence and absence of various concentrations of inhibitors. The initial position of transplanted seedling root tips was marked with a pen on the back of the plates and the root length of 5-15 plants per treatment was subsequently measured at 2-day intervals for an additional 4-5 days. For imaging, roots grown in the presence and absence of inhibitors for 4-5 days were stained with propidium iodide and analyzed by laser scanning confocal microscopy as described below. Inhibitor stocks were prepared as follows. 9 mM brefeldin A (Molecular Probes, Eugene, OR) in dimethylsulfoxide (DMSO); 100 mM caffeine in distilled water (dH₂O); 1 mM latrunculin B in DMSO; 100 mM propyzamide (Chem Service, West Chester, PA) in isopropanol.

Microscopic analysis

For epidermal peels, leaves were attached to microscope slides using double-sided adhesive tape and nonadherent cell layers were scraped away. The remaining epidermal cell layer was washed with dH₂O and incubated with 0.05% w/v Toluidine Blue, 50 mM citrate buffer pH 4.6 with gentle warming (50°C-60°C for about 10-20 seconds).

Transgenic plants expressing the *Arabidopsis* guard cell-specific potassium channel *KATI* promoter β -glucuronidase (*GUS*) gene fusion (*KATI::GUS*) (Nakamura et al., 1995) were kindly provided by M. Sussman (UW-Madison) and were crossed with *scd1-1* plants grown at 16°C. Histochemical assays for *GUS* activity in the F₂ progeny were conducted as described previously (An et al., 1996).

Arabidopsis lines expressing the nuclear-localized N7 and plasma membrane 29-1 GFP-tagged marker proteins (Cutler et al., 2000) were obtained from the *Arabidopsis* Biological Resource Center (Ohio State University, Columbus OH) and were crossed with *scd1-1* plants

grown at 16°C. To visualize guard cell and pavement cell walls, seedlings were stained with 0.1 mg/ml propidium iodide in dH₂O for 5-15 minutes at room temperature prior to imaging. The length of wild-type, *scd1-1* and *scd1-2* epidermal root cells was determined from confocal laser scanning microscopic images of roots that were briefly stained with 0.1 mg/ml propidium iodide for 30 seconds to 1 minute.

Microscopy

Low magnification images were taken with a Nikon CoolPix 950 digital camera. Flower bud and root pictures were obtained with a Leica MZ6 stereo microscope, equipped with a SPOT digital camera (Diagnostic Instruments, Sterling Heights, MI). Higher magnification bright-field images were obtained as described previously (Kang et al., 2001). Confocal laser scanning microscopic (CLSM) images were acquired on a Biorad 1024 confocal microscope (Hercules, CA) equipped with an argon laser using a 63× objective lens. 15-20 frames were averaged for each image. For cryo-scanning electron microscopy, samples were plunge frozen in liquid N₂ transferred to a cryopre-chamber (Emitech K-1150, Houston TX) and sputter coated with gold. Imaging was performed at the CBS Imaging Center (University of Minnesota, St. Paul, MN) in a Hitachi S3500N scanning electron microscope under high vacuum using 5 kV accelerating voltage. For transmission electron microscopy, leaf samples were processed by high-pressure freezing and freeze substitution at the Department of Molecular, Cellular, and Developmental Biology Electron Microscope Service Facility (University of Colorado, Boulder, CO). For improved preservation of *Arabidopsis* shoots, plants were grown on MS-Suc for 4 days and then acclimated over a 2-day period with increasing sucrose concentrations according to the following schedule: 5 ml 3% (w/v) sucrose in dH₂O was added to the MS-Suc plate for 24 hours, followed by addition of 5 ml 5.4% (w/v) (150 mM) sucrose in dH₂O for 24 hours prior to cryofixation. Cotyledon, root tissue and most of the hypocotyl were removed from plants, then the meristematic region, including leaf primordia, was transferred to aluminum sample holders in 150 mM sucrose and frozen in a Baltec HPM 010 high pressure freezer (Technotrade, Manchester, NH), and then transferred to liquid nitrogen. For freeze substitution the samples were incubated in 4% OsO₄ in anhydrous acetone at -80°C for 5 days, followed by slow warming to room temperature over a period of 2 days. The samples were rinsed in several acetone washes, incubated in propylene oxide for 30 minutes, rinsed again in acetone and infiltrated with increasing concentrations of Epon 812-Araldite resin (EM Sciences, Fort Washington, PA) over a period of 8 days and polymerized in 100% resin at 60°C for 2 days. Silver-gold (~80 nm) thin sections were mounted on uncoated copper 200 mesh grids, stained with 8% uranyl acetate in 50% (v/v) ethanol (15 minutes at 60°C) and Reynold's lead citrate (10 minutes at 60°C) and viewed at 80 kV using a Phillips CM120 transmission electron microscope (Eindhoven, Netherlands) at the Medical School Electron Microscope Facility (University of Wisconsin, Madison, WI). All images were processed for publication using Adobe Photoshop 7.0 and Illustrator 10.0 (Adobe Systems, San Jose, CA) software.

Carbon isotope ratios

For isotope ratio measurements of total fixed carbon, plants were grown both under continuous illumination and long days. Analysis was performed in triplicate on dried leaf tissue samples by the University of Utah SIRFER facility (<http://www.ecophys.biology.utah.edu/sirfer.html>).

Oligonucleotides

All oligonucleotides used in this study (Table 1) were designed using the Primer3 software web interface (Rozen and Skaletsky, 2000) (http://www-genome.wi.mit.edu/genome_software/other/primer3.html) and synthesized by Integrated DNA Technologies Inc. (Coralville, IA).

Table 1. Oligonucleotides used in this study

Name	Sequence (5'→3')
P1	catggatccATGGGACGGATCTTCGAGTACTTC
P2	CCACAAGTCATACATCTGCCTG
P3	CATCTATCTTGGGTGGCTACCGC
P4	CACAGCCATTTTGGCAATTCTTTGAC
P5	TAGCAGAGAAAACAAAAGCTTGG
P6	TGACCCTCTAAGTAATGATCTTTAGGC
P7	atagcgccgcACAAAGAAAAGTTGTGGAAGACATAA
SALK LB	CAAACCAGCGTGGACCGCTTGCTGCAACT
UBQFOR	GATCTTTGCGGGAAAACAATTGGAGGATGGT
UBQREV	CGACTTGTCATTAGAAAGAAAGATAACAGG

Lower case letters in the oligonucleotide sequence indicate added restriction sites.

Mapping

The *scd1-1* mutation was mapped in the F₂ population (997 *scd1-1* homozygotes) of a cross between *scd1-1* (Col *gll*) and the Landsberg *erecta* ecotype. Simple sequence length polymorphic (SSLP) (Bell and Ecker, 1994) and cleaved amplified polymorphic sequence (CAPS) (Konieczny and Ausubel, 1993) markers listed in the TAIR database (<http://www.Arabidopsis.org>) and derived from the Cereon SNP database (<http://www.Arabidopsis.org/Cereon/index.html>) (Jander et al., 2002) were used to map *scd1-1* to a 65 kb region on the BAC clone F27J15, containing 18 predicted genes on chromosome I. For each marker used in this study an identifier is listed in Fig. 5A; the Cereon SNP identifier (4xxxxx), TAIR Identifier (F1114SP6) or our identifier (BEDSNP1, BEDSSLP1). The markers we generated have been deposited into the TAIR database: (<http://www.Arabidopsis.org/servlets/TairObject?id=302464&type=marker>) and (<http://www.Arabidopsis.org/servlets/TairObject?id=302463&type=marker>).

Identification of *scd1* mutant alleles

Genomic DNA corresponding to eight of the 18 predicted ORFs in the 65 kb interval containing the *scd1-1* mutation was amplified by PCR. DNA sequence analysis of these PCR products revealed a single point missense mutation (C→T) in the gene At1g49040. Seed stocks for *scd1-2* (SALK_039883) and *scd1-3* (SALK_046851) were obtained from the *Arabidopsis* Biological Resource Center and homozygous *scd1-2* and *scd1-3* seedlings were identified by growth on MS-Suc and confirmed by PCR analysis using T-DNA-specific (Salk Left border) and two *SCD1*-specific (P5 and P6) oligonucleotide primers. Genotype analysis of T-DNA::*scd1* insertion alleles was performed as described previously (Kang et al., 2001) with minor modifications. Separate PCR reactions were performed using primer pairs P5 + P6 and P5 + T-DNA Left border.

Molecular complementation

A 12.8 kb region of genomic DNA containing the entire coding region of At1g49040, 2.3 kb of 5' putative promoter DNA, and 863 bp of 3' flanking untranslated DNA was cloned from the F27J15 BAC DNA into the binary plant transformation vector (pPZP211) (Hajdukiewicz et al., 1994) and transformed using the floral dip method (Clough and Bent, 1998) into homozygous *scd1-1* plants grown at 16°C. Transgenic (T₁) plants were identified on MS-Suc nutrient agar in the absence and presence of 40 µg/ml kanamycin. Upon transfer to soil these plants displayed normal stomatal and floral development when grown at 22°C. T₂ progeny from five independent self-fertilized complemented lines segregated 3:1 [(wild type (*scd1-1/scd1-1*::*SCD1*): *scd1-1/scd1-1*), indicating that the transformants contained a single T-DNA insert that complemented the *scd1-1* phenotype.

RT-PCR analysis and cloning of the *SCD1* cDNA

Total RNA (3 µg) was isolated from various tissues and actively

dividing suspension-cultured cells (T87) (Axelos et al., 1992) using TRI reagent (Sigma Chemical Co., St Louis, MO), reverse transcribed as described (Kang et al., 2001) and diluted 20 fold before PCR amplification. For analysis of *SCD1* expression, a 740 bp product was amplified from the first strand cDNA from each tissue sample using primer pairs P3 + P4, corresponding to a 2270 bp genomic region spanning 9 introns. Genomic DNA and no DNA templates were included in control reactions. Ubiquitin control primers amplify a 484 bp cDNA fragment from nt 1083 to 1567 of the UBQ10 mRNA (At4g05320). DNA sequence analysis confirmed the presence of the C→T transition in the *scd1-1* RT-PCR product amplified with primers P1 and P2, and sequenced with P2.

Full-length *SCD1* cDNA was amplified from first strand cDNA isolated from T87 cells using Proofstart (Qiagen) polymerase (primers P1 and P7) and cloned into the pGEMTeasy vector (Promega, Madison, WI). The 3.5 kb cDNA was sequenced to determine the gene structure and deposited in GenBank (Accession no. AY082605). Three splice site adjustments to the original At1g49040 annotated sequence were made.

RESULTS

Identification and phenotypic characterization of the stomatal cytokinesis-defective mutant *scd1-1*

A recessive stomatal cytokinesis-defective mutant, *scd1-1*, was identified in an ethyl methane sulfonate (EMS)-mutagenized *Arabidopsis* population (Col *gll* background) as described previously (Yang and Sack, 1995) and backcrossed three times with Col *gll* before characterization. The progeny of heterozygous *scd1-1/+* plants segregated 3:1 as expected for a single recessive mutation (627 wt: 206 mutant). The majority (~50-80%) of stomata in the rosette leaves of mature *scd1-1* homozygotes showed severe defects in cytokinesis, including missing or partial ventral cell walls, and cell wall stubs (Fig. 1D-H and Fig. 2B,C). An even higher percentage of cytokinesis-defective stomata (>80%), the majority of which were of the oblate type (Fig. 1H, Fig. 2B,C and Fig. 4D), were observed in the cotyledons and primary leaves of rapidly growing seedlings. Cell wall stubs, which have been observed in other cytokinesis-defective mutants (Söllner et al., 2002), have been proposed to result from a defect in polarized (i.e. asymmetric) cell plate formation in which the cell plate initially fuses to one side of the dividing cell and then grows outward toward the other side of the cell (Cutler and Ehrhardt, 2002; Kang et al., 2003).

The cell division defect observed in the *scd1-1* mutant stomata is not due to problems in guard cell differentiation. Following guard mother cell cytokinesis, a pore normally develops within the newly formed ventral cell wall that separates the two daughter guard cells. Pores were frequently found attached to remaining wall protrusions (i.e. 'hanging pores') in defective guard cells from caffeine-treated wild type and *scd1-1* plants (Fig. 1B, arrows in D,E) indicating that the cytokinesis-defective cells retain guard cell identity. Unattached pores were also occasionally found in mutant cells (i.e. 'torus' Fig. 1F, right). Analysis of the expression of the guard cell-specific reporter gene fusion *KATI-GUS* (Nakamura et al., 1995) (Fig. 1A) confirmed that caffeine-treated (Fig. 1B-C) and *scd1-1* cytokinesis-defective guard cells (Fig. 1G,H) show guard cell identity, demonstrating that the defect in *scd1-1* guard mother cell cytokinesis is not related to a failure in the establishment of stomatal cell fate.

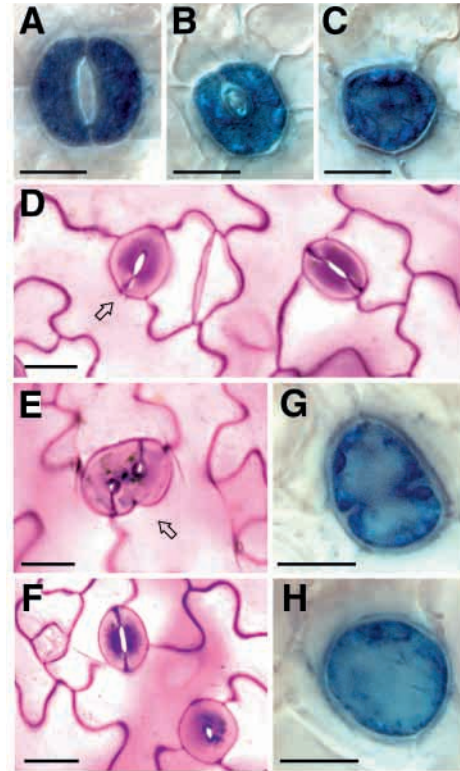


Fig. 1. Wild-type and cytokinesis-defective guard cells. (A-C) Stoma-specific expression of *KATI-GUS*, a marker for guard cell identity (Nakamura et al., 1995), in the cotyledons of 5-day-old seedlings germinated in the absence (A) or presence (B,C) of 5 mM caffeine, an inhibitor of cell plate membrane consolidation. *scd1-1* mutant guard cells phenocopy the effects of caffeine. (D-F) Light microscopy of Toluidine Blue-stained *scd1-1* leaf epidermis from plants grown at 22°C. (G,H) *KATI-GUS* was expressed in cytokinesis-defective *scd1-1* guard cells. Hanging pores are indicated by arrows. A torus and oblate type guard cell are shown in F and H, respectively. Scale bars: 10 µm.

Defective *scd1-1* guard cells were found to be binucleate, confirming that the *scd1-1* phenotype is manifest during cytokinesis. Nuclei were visualized by confocal microscopy in wild type and homozygous *scd1-1* plants expressing a nuclear-localized green fluorescent protein (GFP)-fusion protein marker (Cutler et al., 2000) (Fig. 2). Multinucleated epidermal cells with cell wall stubs were also observed in the leaves of young *scd1-1* seedlings stained with propidium iodide (Fig. 2C) or labeled with the plasma membrane GFP-marker 29-1 (Cutler et al., 2000) (Fig. 2D) demonstrating that the *SCD1* gene product is a general component of the plant cytokinetic machinery. However, no cytokinesis-defective cells were observed in *scd1-1* roots suggesting that requirement for *SCD1* in cytokinesis is likely to vary between plant tissues and cell types.

Although the effects of the *scd1-1* mutation on cytokinesis were only observed in developing guard cells and leaf epidermal cells, the overall growth and development of *scd1-1* mutant plants was severely affected. *scd1-1* plants were smaller than wild type, and displayed defects in seedling development, leaf expansion and flower morphology, which rendered the mutant conditionally sterile (see below). When

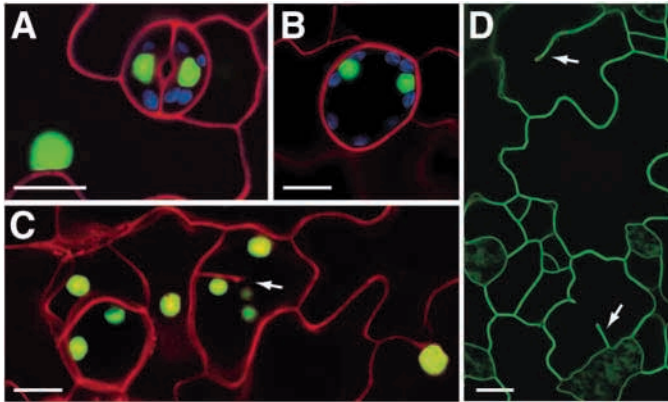


Fig. 2. Defective stomata are binucleate. Cotyledons of 13-day-old wild-type (A) and *scd1-1* seedlings (B,C) that express the nuclear (green) GFP N7 marker and (D) cotyledons from 4-day-old seedlings expressing plasma membrane GFP 29-1 marker (Cutler et al., 2000). Multinucleate *scd1-1* epidermal cells with wall stubs (arrow) are shown in C and D. Two serial confocal optical sections were merged to create C. Cell walls were stained with propidium iodide (red) and chloroplasts are autofluorescent (blue). (D) The membrane in the cell wall stubs of cytokinesis-defective *scd1-1* epidermal cells was contiguous with the plasma membrane. Scale bars: 10 μ m.

grown on a sucrose-containing nutrient agar medium (MS-Suc; see Materials and Methods), the dwarf phenotype of *scd1-1* seedlings was more enhanced (Fig. 3A). Growth of *scd1-1* seedlings on MS-Suc was stunted and the *scd1-1* shoots were

smaller and darker green than wild-type plants (Fig. 3B). *scd1-1* roots were also shorter than wild type (Fig. 3A), and root hairs did not typically elongate. The *scd1-1* mutation resulted in a decrease in the number of cells in the root meristem and an approximately 40% inhibition in the length of cells in the root epidermis and cortex (Fig. 3C, Fig. 8A). *scd1-1* roots, however, did not display a radial swelling phenotype (Fig. 8A) as described for a number of other recently identified mutants with reduced cell elongation (Baskin et al., 1995; Bichet et al., 2001; Nicol et al., 1998; Wiedemeier et al., 2002; Williamson et al., 2001).

As shown in Fig. 4B, flower bud development aborted in the mutants while the buds were quite small (approximately stage 6 of *Arabidopsis* flower development according to (Smyth et al., 1990)). The carpels of defective flowers did not lengthen after pollination, and petals and stamens, two organs that undergo much cell expansion during their development, were rarely observed. Flowering *scd1-1* plants were highly branched, possibly due to a defect in fertility as has been reported previously for male sterile mutants in *Arabidopsis* (Hensel et al., 1994). Microscopic analysis of abnormal *scd1-1* flower sepals showed similar defects in guard cell cytokinesis as those observed in *scd1-1* leaves (data not shown).

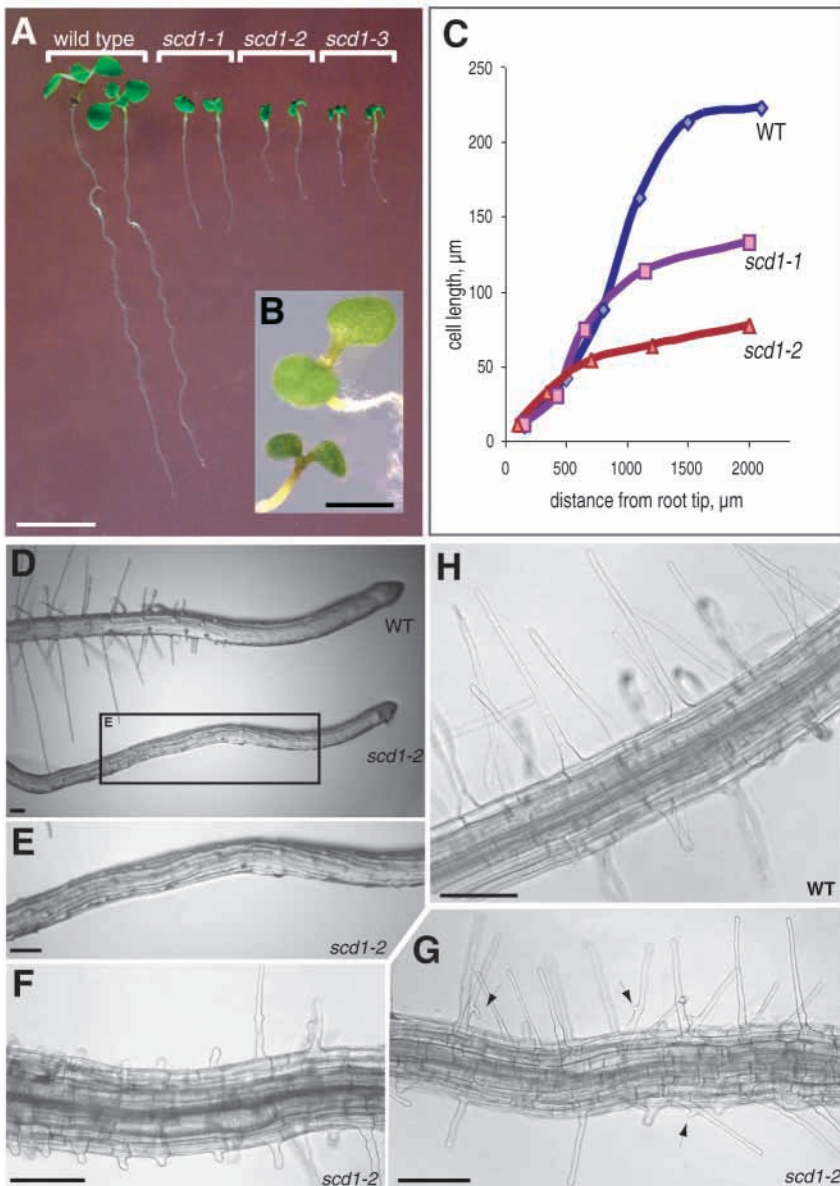


Fig. 3. *scd1* mutants display a dwarfed phenotype. (A) *scd1-1*, *scd1-2* and *scd1-3* mutants grown on MS-Suc under continuous light for 8 days are smaller, have shorter roots, and have darker green leaves than those of wild type. (B) Mutant shoots are smaller and darker green than wild type. (C) Comparison of wild type, *scd1-1* and *scd1-2* root cell size. Cell length was plotted as a function of the distance from the root tip. Each point represents an average of 20-50 cell measurements of a mixture of epidermal and cortical cells. (D-H) Root hair development in wild type and *scd1* mutant seedlings grown vertically on the surface of MS-Suc medium. (D) Stereomicroscope image of surface views of a wild type (WT) and a *scd1-2* root. (E-H) Phase contrast images of (E-G) *scd1/2* and (H) wild-type roots. *scd1* mutants usually form small blebs (D,E,F) at the basal end of trichoblasts, which do not extend. (E) Enlargement of boxed area in D. Arrows in G indicate branched root hairs. Scale bars: 1 cm (A); 0.25 cm (B); 100 μ m (D-H).

The reduction in the number of functional stomata limits gas exchange in the leaves of *scd1-1* mutant plants

The mutant plants are viable most probably owing to the presence of a small population of functional stomata that had undergone normal cytokinesis. To assess how a defect in stomatal formation affects the growth and development of the whole plant, we compared the efficiency of gas exchange in wild type and mutant leaves by analyzing the $^{12}\text{C}/^{13}\text{C}$ isotope ratio of total fixed carbon in mutant versus wild-type plants as described previously (Farquhar et al., 1982). As expected, the $[\text{CO}_2]$ inside the leaf was severely reduced in *scd1-1* mutant plants. The isotopic composition, δ , for wild-type plants was $-30.1 (\pm 0.32, n=6)$ while for *scd1-1* it was $-24.2 (\pm 1.04, n=6)$ corresponding to a $[\text{CO}_2]$ inside the leaf of 259 ppm for wild type and 173 ppm for *scd1-1*. This much lower $[\text{CO}_2]$ inside the leaf will severely reduce photosynthesis in the mutant thereby restricting growth.

scd1-1 is a temperature-sensitive allele

Growth and development of *scd1-1* plants was found to be temperature sensitive. All facets of the mutant phenotype described above were observed in plants grown at 22°C, but were completely absent in plants grown at 16°C (Fig. 4A). When grown at 16°C, all *scd1-1* guard cells appeared normal (Fig. 4E), and the flowers were fully self-fertile (Fig. 4C), making it possible to obtain seed from homozygous *scd1-1* plants. Growth at temperatures higher than 22°C did not result in a more severe phenotype. To address whether the conditional flower morphology defect associated with *scd1-1* plants grown at 22°C was directly related to the *scd1-1* mutation or was an indirect physiological consequence of the lack of functional stomata in the mutant plants, we performed a temperature shift experiment: flowering *scd1-1* plants grown at 22°C were transferred to 16°C for 1 week and then back to 22°C. Morphologically normal self-fertile flowers developed only during the period of growth at the cooler permissive temperature (Fig. 4F), strongly suggesting a direct requirement for *SCD1* function during flower development.

Positional cloning of *SCD1*

Using a map-based approach, we cloned *SCD1* (Fig. 5). A single missense mutation (C→T), consistent with the mutagenic properties of EMS, was revealed in the gene At1g49040 by sequencing of genomic DNA from homozygous *scd1-1*. The mutation resulted in a serine (TCT) to phenylalanine (TTT) change at codon 131 in the predicted open reading frame. We confirmed that At1g49040 corresponds to *SCD1* by molecular complementation as described in Materials and Methods. To determine the genomic structure and organization of the *SCD1* gene, we cloned and sequenced a 3.5 kb cDNA (GenBank Accession no. AY082605). The gene structure of the 9.4 kb *SCD1* gene, which contains 31 exons, is shown in Fig. 5B.

Expression and sequence analysis of *SCD1*

Reverse transcriptase PCR analysis revealed that the *SCD1* transcript was expressed in all organs of the adult wild-type plant tested and in actively dividing *Arabidopsis* suspension-cultured cells (T87) (Fig. 6). *scd1-1* transcript was also found to be expressed in the *scd1-1* mutant suggesting that the

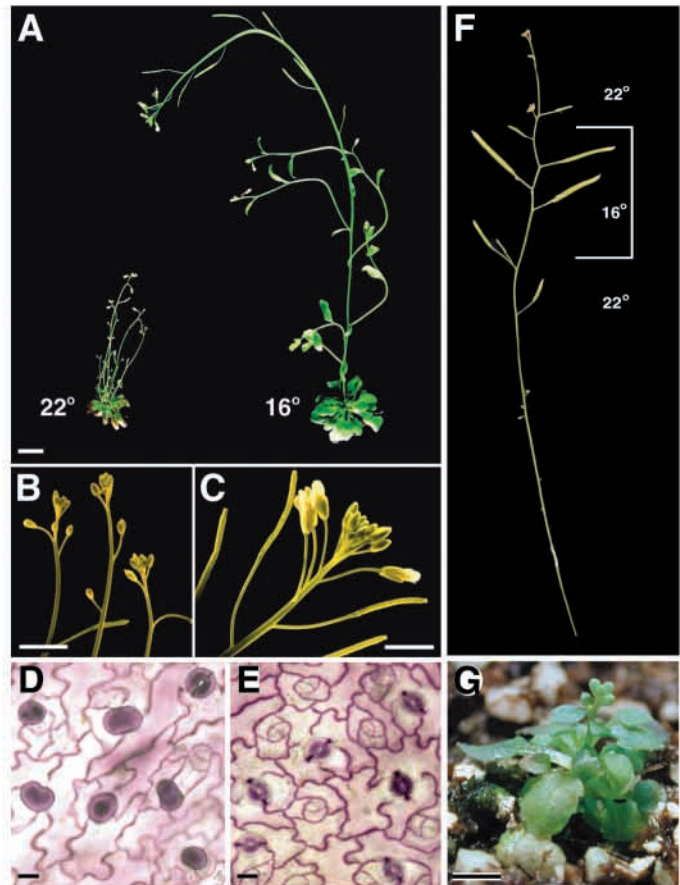


Fig. 4. The *scd1-1* mutant is temperature-sensitive. (A) *scd1-1* plants grown under continuous illumination at 22°C (left) and 16°C (right). (B) Aborted *scd1-1* flower buds from plants grown at 22°C. (C) The fertile inflorescences of plants grown at 16°C. (D,E) Toluidine Blue stained leaf epidermal peels from plants grown at 22°C (D) show many defective guard cells whereas plants grown at 16°C (E) show normal guard cells and an increased number of meristemoids. (F) Temperature-shift experiment. *scd1-1* flower buds that were developing during the 16°C incubation period (bracket) developed into fertile flowers, which produced siliques filled with *scd1-1* seed. Upon return to restrictive conditions (22°C), flower bud development aborted once again. (G) Three-month-old *scd1-3* mutant grown in soil at 18°C. Scale bars: 1 cm (A); 0.5 cm (B,C); 10 μm (D,E); 0.25 cm (G).

missense mutation does not affect *scd1-1* steady-state mRNA levels. RNA samples prepared from 3-day-old T87 cells yielded reproducibly stronger *SCD1* signals both in RT-PCR experiments and RNA blots (data not shown). Although these experiments were not quantitative, they suggest that *SCD1* may be more highly expressed in actively dividing tissue.

SCD1 is predicted to encode an 1187 amino acid soluble protein with a molecular mass of 131.5 kDa. The predicted *SCD1* protein contains an N-terminal DENN domain as well as eight C-terminal WD-40 repeats, which are commonly associated with protein-protein interactions. DENN domains (named for the human DENN protein; Differentially Expressed in Normal and Neoplastic cells) have been found in proteins that interact with regulators of vesicle trafficking and/or other signaling proteins including mitogen-activated protein kinases

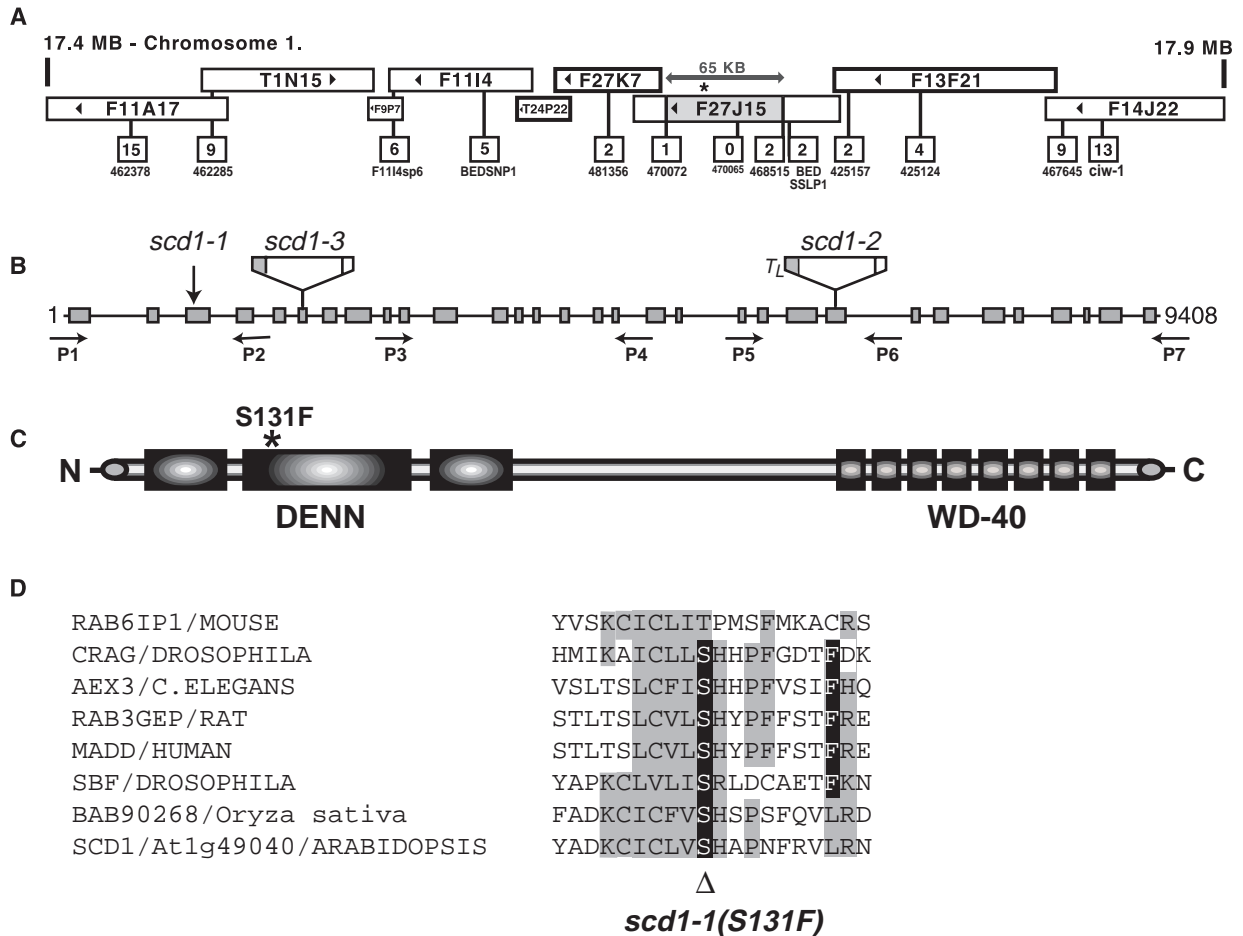


Fig. 5. Identification and organization of *SCD1* and *scd1* alleles. (A) The *scd1-1* mutation was mapped to a 65 kb region on BAC F27J15, containing 18 predicted open reading frames. Vertical lines show marker positions with an identifier number below each line and a box indicating the number of recombinants detected by each marker. (B) The intron/exon map of the *SCD1* gene (At1g49040) in the forward orientation. Positions of the *scd1-1* point mutation and the two T-DNA insertion alleles, *scd1-2* and *scd1-3* are indicated. Arrows denote the location and orientation of primers P1 through P7 described in Table 1 in the Materials and Methods section. TL, T-DNA left border. (C) The domain structure of *SCD1*, with the position of the S131F mutation indicated by an asterisk. The tripartite DENN domain starts at the N terminus of *SCD1* and extends for 430 amino acids (Levivier et al., 2001), the eight WD-40 repeats span a 500 amino acid region extending to the C terminus. (D) Location of the *scd1-1* (S131F) mutation in an alignment of the DENN sub-motif 'F' as identified by hydrophobic cluster analysis (Levivier et al., 2001). The alignment compares the sequence of 6 characterized DENN proteins from various organisms that interact with components of vesicle trafficking and MAPK signaling pathways. Residues shaded in black are identical throughout most of the 43 known and hypothetical proteins identified to date containing DENN domains that are present in the GenBank database. Those shaded in gray are conservative substitutions.

(MAPKs) (Levivier et al., 2001). Most DENN domains are flanked by two additional elements, upstream (u)DENN and downstream (d)DENN, which span several hundred amino acids (Levivier et al., 2001). *SCD1* was found to be the only gene in the *Arabidopsis* genome predicted to encode all three DENN sub-elements. Two other *Arabidopsis* genes of unknown function, At5g35560 and At2g20320, encode proteins containing the uDENN and DENN sub-domains but lacking the dDENN domain which has been suggested to be critical for binding to target proteins, including Rab GTPases (Levivier et al., 2001). The putative At5g35560 and At2g20320 encoded proteins share very limited amino acid sequence identity (<40%) over a short (~65 amino acid) stretch of the *SCD1* DENN domain. The *scd1-1* mutation disrupts a highly conserved serine within the DENN domain (Fig. 5D). Rice

(*Oryza sativa*) has a putative *SCD1* ortholog (GenBank Accession no. BAB90268] that is 68% identical and 81% similar to *SCD1* over the entire length of the protein including the N-terminal DENN and C-terminal WD-40 domains, suggesting that the function of these proteins has been conserved in flowering plant evolution.

***scd1::T-DNA* mutants have severe defects in polarized cell expansion**

To further analyze the function of *SCD1* we isolated and characterized two additional *scd1* alleles. Two independent T-DNA insertion alleles, *scd1-2* and *scd1-3* (Fig. 5B) were identified in the SIGnAL (SALK Institute Genomic Analysis Laboratory) collection of individual sequence indexed T-DNA mutagenized *Arabidopsis* lines (<http://signal.salk.edu/cgi->

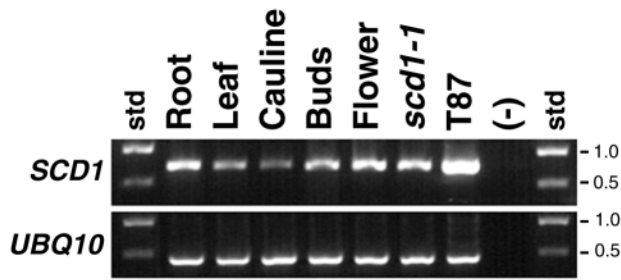


Fig. 6. RT-PCR analysis of *SCD1* expression in wild-type and mutant tissue. RT-PCR analysis of *SCD1* mRNA expression in total RNA isolated from wild-type roots, rosette leaves, cauline leaves, immature flower buds, open flowers, *scd1-1* leaf tissue, and actively dividing suspension-cultured cells (T87). A reaction (-) containing no DNA template was included as a negative control. RT-PCR amplified ubiquitin was used as a loading control. std: 1.0 and 0.5 kb markers.

bin/tdnaexpress). The T-DNA was present in the 23rd exon for *scd1-2*, disrupting the predicted WD-40 repeats of SCD1, and in the 6th exon for *scd1-3*, disrupting the predicted DENN domain. However, *scd1-2* and *scd1-3* plants displayed identical phenotypes, suggesting that these are complete loss-of-function alleles. When germinated on MS-Suc, *scd1-2* and *scd1-3* mutant seedlings were smaller than *scd1-1* seedlings (Fig. 3A), with more severely stunted roots and reduced leaf expansion. In soil, the *scd1-2* and *scd1-3* seedlings produced additional leaves, however the rosettes were more dwarfed than *scd1-1*. In contrast to *scd1-1*, the T-DNA alleles were not temperature sensitive and after a period of three months at 18°C the tiny plants generated defective flowers (Fig. 4G) with buds arresting at similar developmental stage as observed in *scd1-1* plants grown at 22°C (Fig. 4B).

Further morphological and molecular characterization was performed on the *scd1-2* T-DNA-induced mutation. *scd1-2* segregated as a single recessive gene (a heterozygous *scd1-2* plant segregated 384 wild type:121 dwarf). PCR genotype analysis of segregating *scd1-2* seedlings confirmed that the dwarfed plants (Fig. 3) were homozygous for the T-DNA insert (data not shown). To test for allelism, fertile homozygous *scd1-1* plants were crossed with *scd1-2* heterozygotes. Progeny of several independent reciprocal crosses segregated approximately 1:1 for *scd1* and wild-type phenotype seedlings when grown on MS-Suc medium, confirming that *scd1-1* and *scd1-2* are allelic.

Stomatal development was severely disrupted in *scd1-2*. Morphologically identifiable guard cells or guard mother cells were virtually absent in the leaf epidermis (Fig. 7B,F,G). Therefore the defect in guard cell development in the *scd1-2* mutant is more severe than in the weaker *scd1-1* allele. Leaf epidermal pavement cell shape was also altered in *scd1-2* seedlings (Fig. 7A,B). In wild-type leaves and *scd1-1* mutants these cells have sinuous cross-walls with interdigitating lobes and valleys giving a 'jigsaw puzzle'-type appearance to the leaf surface (Figs 1, 2 and Fig. 7A). In contrast, *scd1-2* (Fig. 7B) and *scd1-3* (data not shown) pavement cells were less expanded in all directions than wild-type cells, and the cell walls were less lobed giving the mutant cells a more rectangular appearance. Small gaps were also observed between mutant pavement cells (Fig. 7F).

Trichome morphogenesis was also severely inhibited in the T-DNA::*scd1* insertion lines. *Arabidopsis* leaf trichomes are branched unicellular structures projecting outward from the epidermis. *scd1-2* and *scd1-3* trichomes developed only to the stage of unbranched blebs (Fig. 7D,E). These abnormally developing trichomes were mechanically unstable and ruptured during leaf development (Fig. 7D-G). Craters were frequently found in the center of a group of subtending socket cells, where the trichome would normally be found (Fig. 7D,F,G). The trichomes of the F₂ progeny of a cross between *scd1-1* and wild-type Col *GL1* appeared normal (data not shown).

The epidermal and cortical cells of *scd1-2* mutant roots were approximately 1/3 the length of wild type cells and like *scd1-1*, there were fewer cells within the root division zone (Fig. 3C, Fig. 8A). Root hair formation was usually arrested in all *scd1* mutants at the transition to tip growth stage of development (Parker et al., 2000), resulting in short bulges in the basal end of trichoblasts (Fig. 3D-F, *scd1-2* shown). Under certain conditions, however, all *scd1* alleles formed root hairs that were significantly shorter (≤ 150 μm in length) than those of the wild type (Fig. 3F-H). These root hairs appeared to form under growth conditions that have previously been reported to cause a stress-induced pathway of root hair development (Okada and Shimura, 1994). In particular, *scd1* root hair expansion was observed when seedlings were grown on MS-Suc containing high agar concentrations (1 to 1.5% w/v), which prevented adequate root contact with the surface of the growth medium. In contrast to fully expanded wild-type root hairs (Fig. 3H), abnormal bulges and branches were observed on stress-induced *scd1-2* root hairs (Fig. 3F,G).

Electron microscopic analysis of *scd1* mutant cells

To address whether a loss of the SCD1 protein affected membrane trafficking we analyzed cytokinesis-defective *scd1* leaf epidermal cells by transmission electron microscopy. For studying cell plate biogenesis, ultrarapid cryofixation is superior to chemical fixation, which may cause artifactual vesiculation of this dynamic membrane system (Otegui and Staehelin, 2000; Otegui et al., 2001; Samuels et al., 1995; Samuels and Staehelin, 1996). Thin sections of wild-type and *scd1-2* leaf primordia (<0.5 mm in length) were prepared from high-pressure frozen, freeze substituted material. Fig. 7H shows a dividing wild-type cell with an expanding cell plate at the fenestrated sheet stage. Several vesicles can be observed near the leading edge of the developing cell plate as it grows toward the cell cortex. In contrast, a cluster of secretory vesicles containing electron dense cell wall material was observed near the tip of the cell wall stub of a cytokinesis-defective *scd1-2* cell (Fig. 7I). Similar defects in vesicle accumulation and cell plate maturation have been observed in cytokinesis-defective cells of *knolle* and *keule* embryos (Lauber et al., 1997; Waizenegger et al., 2000).

Inhibitor studies

To further analyze the function of SCD1 we examined the effects of several membrane trafficking inhibitors and cytoskeletal antagonists on cell expansion and cytokinesis in *scd1* mutant and wild-type root cells. The inhibitors used in these studies include caffeine (blocks cell plate consolidation), brefeldin A (BFA; inhibits vesicle secretion), latrunculin B (Lat B; microfilament (MF)-depolymerization), and propyzamide

(microtubule (MT)-destabilization). We used roots for these studies because they can be grown in direct contact with the inhibitors and because their very regular pattern of cell files make them highly amenable to imaging. To compare the effects of the various inhibitors on root growth, we first grew the

Arabidopsis seedlings for several days on MS-Suc in the absence of the inhibitors, transplanted them to medium containing the drugs and then measured the extent of root growth over a 5-day period. Shown in Fig. 8 are representative wild-type and *scd1* root tips from the various inhibitor

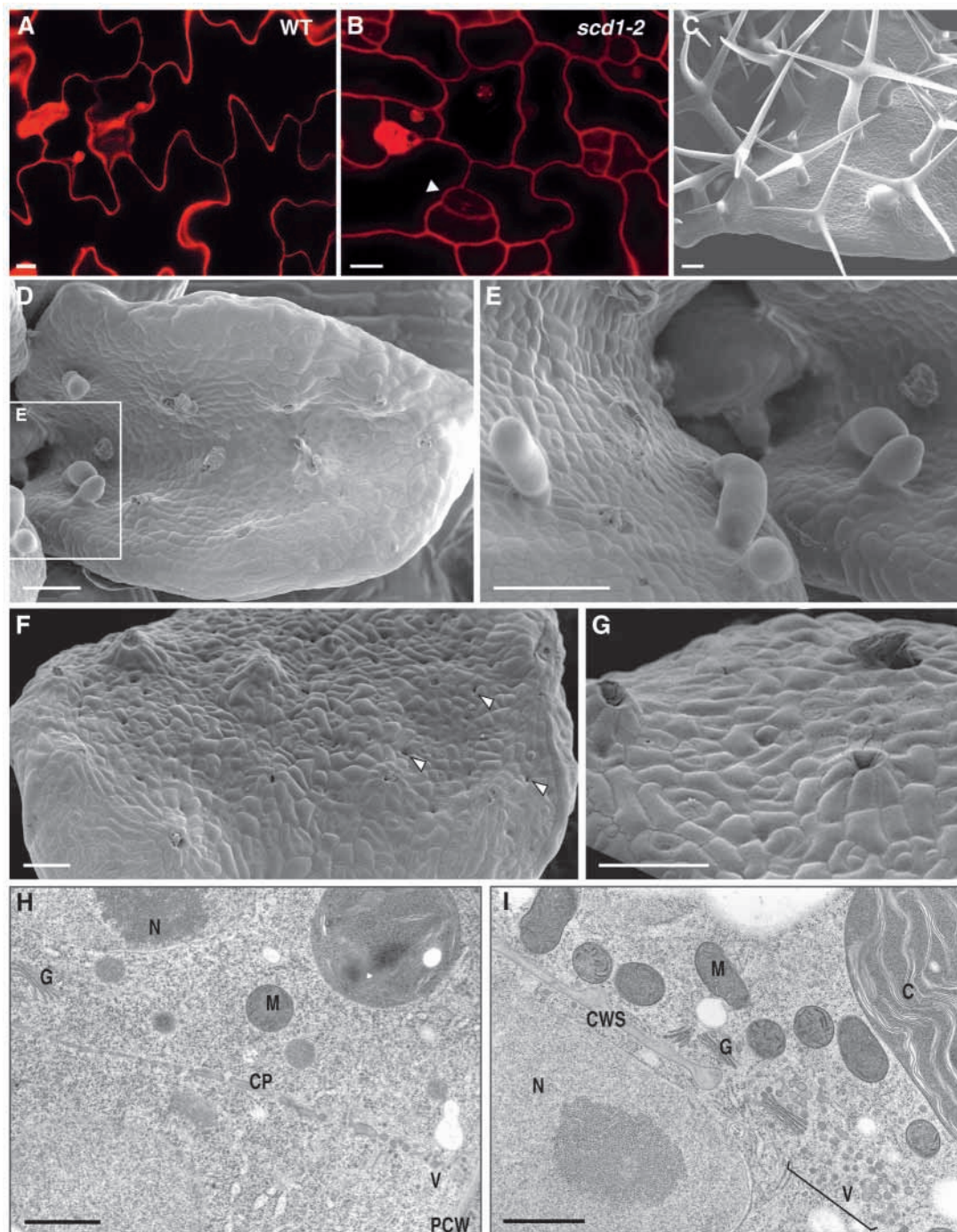


Fig. 7. *scd1::T-DNA* mutants showed defects in leaf trichome and epidermal pavement cell expansion. (A) wild type and (B) *scd1-2* cotyledon epidermal cells stained with propidium iodide. The white arrowhead indicates a potential recently divided guard mother cell. (C) Wild-type and (D-G) *scd1-2* leaf trichomes on emerging leaves. (E) An enlarged view of expanding unbranched *scd1-2* trichome initials. (F,G) Older leaves of *scd1-2* seedlings showing mechanically unstable trichomes and small gaps between pavement cells (arrowheads). Cell debris is often observed in craters surrounded by the subtending socket cells of broken trichomes. (H,I) Transmission electron micrographs of cells from very young leaves; (H) a wild-type telophase cell and (I) a cytokinesis-defective *scd1-2* cell. C, chloroplast; CP, cell plate; CWS, cell wall stub; PCW, parental cell wall; G, Golgi; M, mitochondrion; N, nucleus; V, secretory vesicles. Scale bars: 10 μ m (A,B); 50 μ m (C-G); 1 μ m (H,I).

treatments and root length dose-response curves for each set of drug treatments. Although the untreated *scd1* root tips (Fig. 8A) appear slightly narrower than wild-type ones, no significant differences in the overall diameter of wild type, *scd1-1* and *scd1-2* roots were routinely observed (Fig. 3D-G).

The difference observed in *scd1* versus wild-type root length (Fig. 3A) is due not only to an inhibition in anisotropic cell expansion (Fig. 3C) but also to a reduction in cell production. Analysis of the growing tips of wild-type, *scd1-1* and *scd1-2* roots revealed that there are fewer cells in the division and transition zones of both mutants compared to wild type (Fig. 8A). In wild-type roots the division and transition zones (from the meristem to the start of the elongation zone) contained approximately 30-50 cells in the epidermal or cortical cell files, whereas *scd1-1* roots contained 10-15 and *scd1-2* roots contained 7-10 cells, respectively. All of the drug treatments we tested reduced the size of the transition zone in both wild type and mutants, resulting in the initiation of cell expansion immediately adjacent to the meristem in the *scd1* roots.

Wild-type root cells were extremely swollen and

disorganized when grown in the presence of 8 μM propyzamide (Fig. 8E). The mutant root cells in comparison were moderately swollen, and the roots less affected than wild type with respect to changes in their general shape, organization and diameter. The MT-stabilizing drug taxol (paclitaxel) caused similar effects on root elongation and cell expansion as observed for propyzamide (data not shown).

scd1 root cell morphology was more sensitive to the effects of caffeine, BFA and Lat B than wild type. No differences in the degree of root growth inhibition, relative to the no drug control, were observed between mutants and wild type. Rather, the cells in *scd1* root tips became more disorganized than in wild type in the presence of caffeine, BFA and Lat B (Fig. 8B,C). As described above, cytokinesis-defective cells were not detected in *scd1-1* and *scd1-2* roots. However, in the presence 1.2 mM caffeine we observed an increase in the number of *scd1* root cells containing one or more cell wall stubs relative to wild type (Fig. 8B). Cell wall stubs were also occasionally observed in mutant roots treated with BFA and Lat B. Lat B caused a slight increase in radial growth of

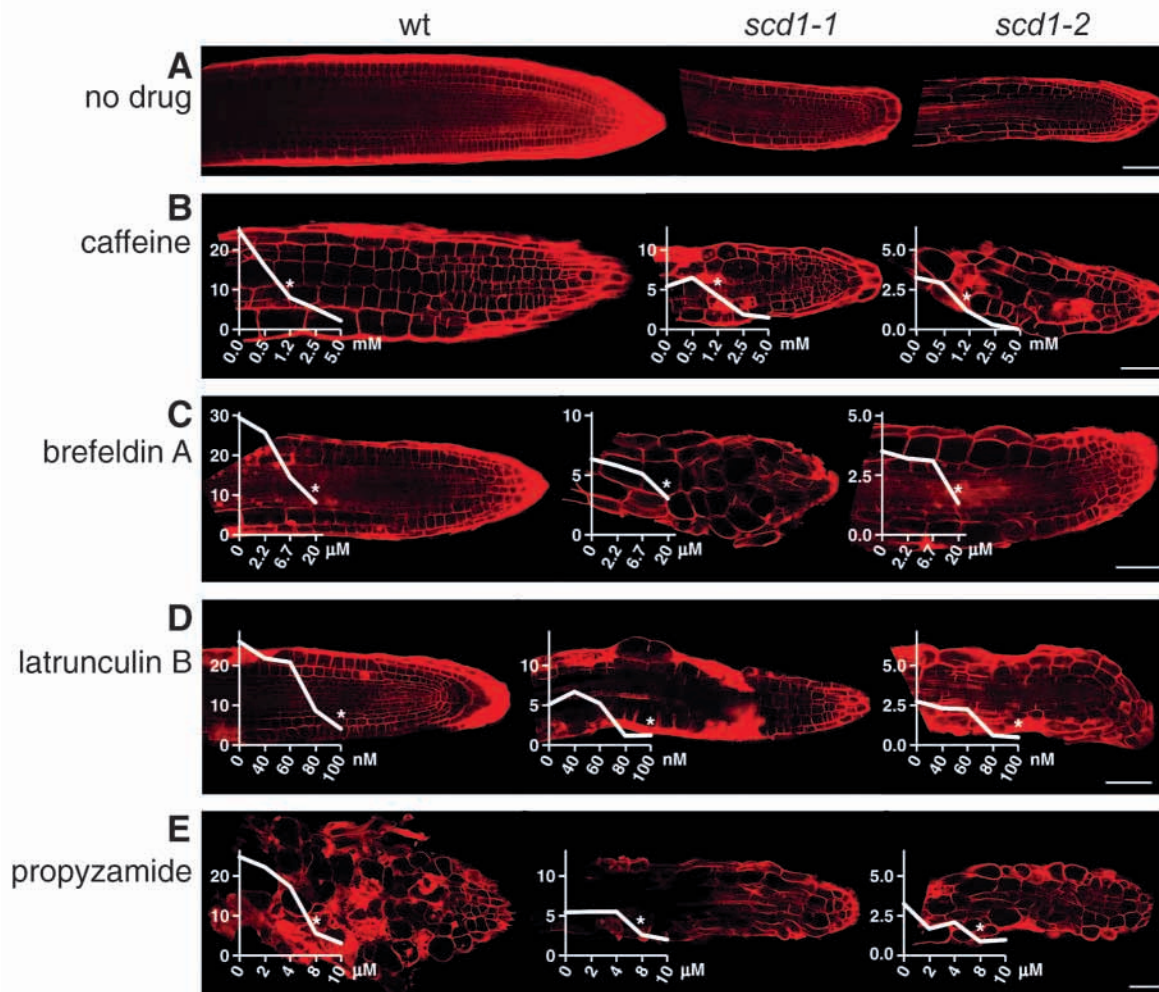


Fig. 8. Effects of vesicle and cytoskeletal inhibitors on wild type and *scd1* root growth and cell morphogenesis. CLSM optical sections of wild type (wt), *scd1-1* and *scd1-2* roots grown in (A) the absence and (B-E) the presence of caffeine (B), brefeldin A (C), latrunculin B (D) and propyzamide (E). The graph associated with each image represents the extent of root growth (y-axis; mm) over a 4- to 5-day period in the absence and presence of the inhibitors at the indicated concentrations (x-axis). The asterisk denotes the inhibitor concentration used in the underlying root section. 5-15 seedlings were measured for each treatment and genotype. Scale bars: 50 μm .

elongating *scd1* root cells. In untreated *scd1-2* roots we have occasionally observed swollen epidermal cells. The frequency of these abnormal cells increased in both *scd1-1* and *scd1-2* roots grown in the presence of Lat B.

DISCUSSION

In this paper we describe the isolation of a unique plant gene, *SCD1*, that is required for cytokinesis and cell expansion. Recent studies have suggested that the analysis of mutants displaying weaker cytokinesis-defective phenotypes compared to seedling and embryo-lethal mutants (such as *knolle* and *keule*) would reveal additional components of the plant cytokinesis machinery (Müller et al., 2002; Söllner et al., 2002; Yang et al., 1999). The conditional mutant, *scd1-1*, was identified in a screen for mutants that affect stomatal development. This screen was chosen because cytokinesis-defective guard mother cells are readily observed because of their highly stereotypical morphology. *scd1-1* mutants also exhibit defects in leaf pavement cell cytokinesis. Cytokinesis-defective stomatal and leaf pavement cells in *scd1-1* are multinucleate, often with cell wall stubs, indicative of arrested cell plate formation.

A novel feature of the *scd1-1* plants is their temperature-sensitive phenotype. At the restrictive temperature of 22°C, *scd1-1* mutants display obvious defects in leaf cell cytokinesis. In addition, mutant plants are dwarfed and show defects in floral organ and root morphogenesis. Previous studies have shown that defects in cell division and expansion affect normal floral organogenesis (Hauser et al., 1998); however, the cellular basis for the abnormal flowers in the *scd1-1* plants remains to be characterized. At lower (i.e. permissive) temperature, the *scd1-1* gene product appears to retain greater activity, as *scd1-1* plants were fully fertile and stomata were completely normal. The results of our temperature-shift experiments (Fig. 4) suggest that the missense mutation in the DENN domain of *scd1-1* is a temperature sensitive partial loss-of-function mutation. *scd1-2* and *scd1-3* plants, which are not temperature-sensitive, displayed more severe phenotypes than *scd1-1* under all growth conditions and are thus likely to be complete loss-of-function mutants. The temperature-sensitive nature of the *scd1-1* mutation should aid the molecular characterization of SCD1 function.

Conditions that promote rapid growth (e.g. the presence of exogenous sucrose) enhanced the severity of the *scd1* phenotype. During rapid growth, *scd1-1* stomata were primarily the oblate type, which lack cell wall stubs and pores, suggesting that disruption of cell plate assembly in the mutant guard mother cells had occurred very early during cytokinesis.

Role of SCD1 in cytokinesis and polarized cell expansion

During cytokinesis, exocytic vesicles carrying membrane and cell wall material are targeted toward the division plane to assemble the cell plate. Likewise, plant cell expansion, which controls cell shape and ultimately plant morphology, is accomplished by polarized membrane trafficking and localized release of secretory pathway-derived membrane and cell wall material at specific sites on the plasma membrane.

Characterization of two membrane-trafficking factors, KEULE (Söllner et al., 2002) and the *Arabidopsis* dynamin-related protein, ADL1A (Kang et al., 2003) has recently shown that common molecular components of the membrane-trafficking machinery are used during polarized cell expansion and cytokinesis. Similarly, sequence analysis and phenotypic characterization of the *scd1* mutants suggest that SCD1 is required for vesicular trafficking during cell plate formation as well as for the polar growth of various plant cell types.

SCD1 encodes a unique *Arabidopsis* protein containing two domains that are likely to be critical for function, an N-terminal DENN domain and a series of C-terminal WD-40 repeats that may mediate protein-protein interaction. DENN domains have been identified in several proteins that associate with members of the Rab family of small GTP-binding proteins, which regulate membrane trafficking. Although the precise function of DENN domains remains to be defined, its presence in the Rab3 guanine nucleotide exchange factor (GEF) from *Drosophila* (Shirataki et al., 1994), rat (Oishi et al., 1998) and *C. elegans* (Iwasaki and Toyonaga, 2000) and the mammalian Rab6 interacting protein, Rab6IP1 (Janoueix-Lerosey et al., 1995) has led to the suggestion that DENN domain-containing proteins may regulate Rab activity (Levivier et al., 2001).

Each branch of the secretory pathway is thought to contain a specific Rab that functions to coordinate targeting and docking of transport vesicles to their appropriate acceptor membranes (Rutherford and Moore, 2002). Inhibition of Rabs and their accessory proteins, including GEFs that function in Golgi to plasma membrane transport, has been shown to lead to the accumulation of unfused secretory vesicles in yeast (Novick et al., 1981; Novick et al., 1980; Novick and Schekman, 1979). Similarly we have observed the accumulation of unfused vesicles in the cytokinesis-defective *scd1-2* leaf cell shown in Fig. 7I. Consistent with its potential role in regulating membrane trafficking to the cell plate and cell surface, our preliminary localization and subcellular fractionation studies indicate that SCD1 is a tightly-associated peripheral membrane protein associated with the plasma membrane and vesicular structures (T.G.F., L.M.K. and S.Y.B., unpublished data).

As shown in yeast and mammalian cells, it is likely that multiple exocytic routes mediate the polarized trafficking of proteins and cell wall polysaccharides to the plant cell surface and division plane during cytokinesis (for review see Bednarek and Falbel, 2002). Yeast mutants lacking only one of two exocytic pathways remain viable but grow more slowly (Gurunathan et al., 2002). Likewise, cell plate biogenesis and cell expansion may not be fully blocked in the *scd1* mutants because other SCD1-independent trafficking routes may partially compensate for the loss of SCD1. Consistent with a role for SCD1 in membrane trafficking, *scd1* root cell cytokinesis was more sensitive to low doses of caffeine, an inhibitor of cell plate consolidation (Samuels and Staehelin, 1996) and to BFA, a general inhibitor of vesicle formation. At the concentrations of BFA used in these experiments plant Golgi remain intact but secretion is inhibited (Geldner et al., 2001; Staehelin and Driouich, 1997). However, we cannot exclude the possibility that changes in drug uptake as well as changes in the overall physiology of *scd1* plants may affect their response to the various inhibitors we have tested. Therefore it will be necessary to further test the role of SCD1

in membrane transport through additional biochemical and genetic experiments.

We hypothesize that SCD1 regulates the function of a Rab GTPase required for targeting and fusion of one class of secretory vesicles during cell plate formation and cell expansion. The loss of SCD1 therefore reduces the efficiency of membrane and cell wall material secretion necessary for rapid cell expansion and could, in certain cells, cause defects in cell plate assembly. Because the precise role of only a few plant Rabs have been experimentally defined to date, it is premature to speculate which of the fifty-seven Rabs encoded in the *Arabidopsis* genome (Rutherford and Moore, 2002) could potentially be the target of SCD1. The enhanced radial cell expansion defects observed upon treating *scd1* root cells with BFA or Lat B could result from reduced secretion of cell wall precursors and biosynthetic enzymes necessary to maintain cell wall integrity and constrain the anisotropic direction of cell growth. As shown in Fig. 7, expanding *scd1-2* trichomes are indeed mechanically unstable and rupture prior to the initiation of branching. This phenomenon may be similar to that observed in the root hairs of the defective cellulose synthase-like protein mutant, *kojak* (Favery et al., 2001), which burst after initiation.

Vesicle trafficking and cytoskeletal dynamics are clearly interrelated processes whose activity must be coordinated to establish proper cell shape. For example, BFA has been shown to inhibit the proper localization of ROP GTPases, which regulate actin dynamics during cell expansion (Molendijk et al., 2001). Interestingly, the leaf pavement cells of transgenic plants expressing a dominant-negative GDP-bound mutant form of ROP2 were smaller and had fewer lobes (Fu et al., 2002) similar to *scd1-2*. Although our data suggest that SCD1 functions primarily in membrane trafficking, loss of SCD1 may also have an effect on the plant cytoskeleton. We have found by immunofluorescence microscopy, using anti-tubulin antibodies, that the cortical, mitotic spindle and phragmoplast MTs were organized normally in *scd1* root cells (data not shown). Our inhibitor studies, however, indicated that *scd1* root cell expansion was less sensitive than wild type to MT antagonists. One possible explanation for this is that MT dynamics may be affected by the rate of cell plate and cell surface expansion.

In addition to its role in membrane trafficking, SCD1 may also be involved in other signaling pathways required for cell expansion and cytokinesis. For example, proteins containing DENN-domains such as the mammalian mitogen-activated kinase activating death domain protein (MADD) are also involved in the regulation of MAPK signaling pathways in addition to their function in Rab-mediated processes (Majidi et al., 1998; Schievella et al., 1997). Evidence for a role of DENN-containing proteins in both signaling pathways also comes from the study of the *C. elegans* Rab3 GEF, AEX-3, which is highly related to the human MAPK interacting DENN-protein, MADD. *aex-3* mutants display additional defects not observed in *rab-3* mutants, indicating that this Rab3 GEF functions in both vesicular trafficking and other signaling pathways (Iwasaki et al., 1997; Iwasaki and Toyonaga, 2000).

MAPK signaling pathways are involved in plant cytokinesis and cell expansion (Nishihama et al., 2002; Samaj et al., 2002). Interestingly, defects in the tobacco MAPK kinase kinase (MAPKKK), NPK1, and the *Arabidopsis* NPK1 orthologs,

ANP2, ANP3 (Jin et al., 2002; Krysan et al., 2002; Nishihama et al., 2001) resulted in the development of dwarfed plants that display stomatal cytokinesis defects similar to *scd1-1* (Nishihama et al., 2001); T.G.F., P. J. Krysan and S.Y.B., unpublished data). In addition, the stress-induced MAPK (SIMK in *Medicago* and *Nicotiana*, homologous to MPK6 in *Arabidopsis*) has recently been shown to play an important role in actin-dependent root hair elongation (Samaj et al., 2002). Analysis of the phenotypes of *scd1 anp2 anp3* triple and *scd1 mpk6* double mutants will help to determine if SCD1 and the MAPKs function in the same or in parallel signaling pathways to regulate cytokinesis and cell expansion.

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