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SCHIZORIZA controls an asymmetric cell division and restricts epidermal identity in the *Arabidopsis* root

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SUMMARY

The primary root of *Arabidopsis* has a simple cellular organisation. The fixed radial cell pattern results from stereotypical cell divisions that occur in the meristem. Here we describe the characterisation of *schizoriza* (*scz*), a mutant with defective radial patterning. In *scz* mutants, the subepidermal layer (ground tissue) develops root hairs. Root hairs normally only form on epidermal cells of wild-type plants. Moreover, extra periclinal divisions (new wall parallel to surface of the root) occur in the *scz* root resulting in the formation of supernumerary layers in the ground tissue. Both *scarecrow* (*scr*) and *short root* (*shr*) suppress the extra periclinal divisions characteristic of *scz* mutant roots. This results in the formation of a single layered ground tissue in the double mutants. Cells of this layer develop root hairs, indicating that mis-specification of the ground tissue in *scz* mutants is uncoupled to the cell division defect. This suggests that during the development of the ground tissue *SCZ* has two distinct roles: (1) it acts as a suppressor of epidermal fate in the ground tissue, and (2) it is required to repress periclinal divisions in the meristem. It may act in the same pathway as *SCR* and *SHR*.

Key words: Root, Radial pattern, Epidermis, Root hair, *schizoriza*, *Arabidopsis thaliana*

INTRODUCTION

In plants, meristems are populations of stem cells from which the body of the plant subsequently develops. Cells within the primary shoot meristem give rise to different organs such as leaves, stem and the floral organs. The primary root meristem, however, elaborates a single organ, the root, which has a stereotyped radial pattern of tissues. In *Arabidopsis* this radial pattern is simple. Root cells are organised as concentric rings of lateral root cap, epidermis, ground tissue (cortex and endodermis), and a pericycle surrounding a central stele (Dolan et al., 1993) (see Fig. 4A). This cell pattern is first laid down during embryogenesis and is maintained by regular divisions of stem cells (initials) in the meristem of the developing root. These initials surround four central cells (quiescent centre) that divide infrequently. There are four sets of initials: the lateral root cap/epidermal initials, the cortical/endodermal initials, the columella initials and the pericycle/vascular initials (Dolan et al., 1993). Initials divide in a stereotypical pattern to give rise to the cells in each of the tissue layers (see Fig. 4A). For example, the cortex/endodermis initial divides anticlinally (new wall perpendicular to the root surface) to generate a new initial and a daughter cell. The daughter cell divides periclinally (new wall parallel to the surface of the root) and asymmetrically to generate a small inner cell which is the daughter cell of the endodermis and a larger outer cell that will develop as cortex. Initials divide slowly, adding to the population of rapidly dividing cells in the meristem. After division, cells elongate before they acquire their mature size and shape in the differentiation zone. Once the radial organisation of tissues is established in the root, the tangential differentiation of cell types occurs in the epidermis. The mature epidermis is composed of cells bearing hairs that develop from meristematic trichoblasts, and non hair-bearing epidermal cells which develop from atrichoblasts.

The identification of mutants with defects in radial pattern has provided a basis for understanding the mechanism of radial patterning in the root. Plants homozygous for the *scarecrow* (*scr*) and *short root* (*shr*) mutations have defects in the division of the cortex/endodermis initial daughter cell resulting in the formation of a single layer of ground tissue instead of two (Benfey et al., 1993; Scheres et al., 1995). *SCR* is essential for cell division, but not differentiation, of the ground tissue (Scheres et al., 1995; Di Laurenzio et al., 1996). In contrast, *SHR* plays a role in both cell division and the differentiation of the endodermis (Benfey et al., 1993; Helariutta et al., 2000). *SCR* and *SHR* are members of the GRAS family of transcription factors (Di Laurenzio et al., 1996; Helariutta et al., 2000). *SCR* is expressed in the quiescent centre, the cortex/endodermis initial and the endodermis (Di Laurenzio et al., 1996; Wysocka-Diller et al., 2000). *SHR* is expressed in the stele (pericycle and vascular cylinder) and has been shown to act in a non cell-autonomous manner (Helariutta et al., 2000). Genetic analysis has shown that *SCR* and *SHR* act in the same

pathway since *shr* is epistatic to *scr* and SHR is required for *SCR* transcription.

Here we describe a mutant called *schizoriza* (*scz*) which was identified in a screen for genes involved in the development of the root epidermis. Plants homozygous for the *scz* mutation develop supernumerary ground tissue layers and root hairs emerge from subepidermal cells. The extra periclinal divisions that occur in *scz* mutants are suppressed by both *scr* and *shr* mutations indicating that *SHR*, *SCR* and *SCZ* act in the same pathway in the regulation of cell division in the root meristem. Because *scz scr* and *scz shr* double mutants do form root hairs in the ground tissue it suggests that the mis-specification of subepidermal cell fate by *SCZ* is independent of *SHR* and *SCR*.

MATERIALS AND METHODS

Plant growth conditions

Seeds were surface sterilised in 5% sodium hypochlorite and sown on half strength Murashige and Skoog (Duchefa, Haarlem, The Netherlands) medium (pH 5.8), containing 1% sucrose and 0.8% phytagel. The plants were stratified for 2 days and grown in continuous light conditions at an angle of 45°.

Mapping the *schizoriza* mutation

Col and Col-0 plants were used to pollinate homozygous *scz* mutant plants (ecotype L*er*). An F_2 population was generated. Individual F_2 *scz* mutants were selected for mapping. DNA isolated from the mutants was analysed using CAPS and SSLP markers (Klimyuk et al., 1993; Konieczny and Ausubel, 1993; Bell and Ecker, 1994).

Confocal microscopy

Three- to 7-day-old seedlings were stained with 0.1 mg/ml propidium iodide solution for 5-15 minutes. Propidium iodide-stained roots were imaged with an MRC1024 Biorad confocal microscope using 568 nm excitation line and a YHS filter block or a Leica TC5 SP confocal microscope using the 568 nm excitation and 498-551 nm emission lines. The 488 nm excitation and 580-700 nm emission lines on the Leica microscope were used to image GFP expression in the enhancer trap lines. Images were processed using NIH Image (http://rsb.info.hih.gov/nih-image) and assembled using Adobe Photoshop 5.

Crosses with marker lines

Several GFP enhancer trap lines from the Haseloff collection were crossed into the *scz* background. J0481 is expressed in all epidermal cells from the elongation and differentiation zones and the lateral root cap cells (lrc). J0672 and J2092 are expressed in all epidermal cells from the elongation and differentiation zones at 3 days after germination, and by 5 days they are only expressed in atrichoblasts. These lines are also expressed in the lrc. J0571 is expressed in the quiescent centre, the cortex/endodermis initial and daughter cells, and all the cells of the ground tissue in the meristem, elongation zone, and differentiation zone. J3611 is expressed in endodermal cells in the differentiation zone and J2672 is expressed in the endodermis from the endodermal daughter cell into the elongation and differentiation zones. J2672 is also expressed in lateral root cap cells. J2931 and Q2393 are expressed in the lateral root cap, epidermis and cortex. N9173 is expressed in the epidermis/lateral root cap daughter cells, in the lateral root cap cells, and in all the epidermal cells of the meristem. In the elongation zone it is expressed in the atrichoblasts of the epidermis. In the differentiation zone, it is expressed in the atrichoblasts and the expression spreads to all cells of the ground tissue, the pericycle and the vasculature.

Number of plants used for the analysis

Fifty *scz* mutant roots were analysed with confocal microscopy at 3 days, 4 days and 5 days after germination. Thirty wild-type plants and 30 *scz* GFP-expressing plants were examined using confocal microscopy at 3 days, 5 days and 7 days after germination (10 plants at each time point) for each GFP enhancer trap line. Several hundred plants from each double mutant combination were plated and observed with a stereomicroscope. Thirty plants from each double mutant combination were analysed further with confocal microscopy. 25 *scz GL2::GUS* plants were stained for GUS activity. Six *scz GL2::GUS* plants embedded in Technovit, were sectioned to look for GUS activity in subepidermal positioned cells.

Semi-quantitative RT-PCR analysis

Total RNA was isolated from L*er* and *scz* 3-day-old roots using an RNeasy™ Plant Mini Kit (Qiagen). Genomic contamination was removed by DNase treatment. 3 μg of RNA was reverse transcribed with oligo(dT) using murine reverse transcriptase and used as templates for PCR amplification. The gene specific primers SCR5: 5′-GGAATTTACGCGGCTTTGCCTTCACGGTGGATG-3′ and SCR3: 5′-TACAAATCTTCCTAAGAAAGAACCAGCGTGGCT-3′, were used to amplify *SCR*. These primers produce a fragment of 564 bp when cDNA is used as a template and a fragment of 680 bp when genomic DNA is the template. Primers EF1 5′-GCTCTATGGAAG-TTCGACC-3′ and EF2 5′-GGTGTGGCAATCGAGAACTGGG-3′ were used to amplify the *Arabidopsis* elongation factor 1-alpha 4 (Liboz et al., 1989), for a control of equal amounts of cDNA used in the PCR reactions. These primers produce a fragment of 811 bp when cDNA is used as template and a fragment of 912 bp when genomic DNA is used. PCR reactions were run for 22, 25, 28, 31 and 34 cycles for *SCR* and 18, 22 and 25 for *EF1*.

RESULTS

Identification and genetic analysis of the *schizoriza* root mutant

A population of Ac/Ds transposon tagged lines was visually screened to isolate root mutants with defects in epidermal development. The screen identified a mutant that developed hairier roots than wild-type plants. The mutant was named *schizoriza* (*scz*). Apart from a decrease in shoot stature the mutant displayed no other obvious aberrant phenotype. The *scz* mutation segregates as a single recessive Mendellian factor (χ^2=1.05), and maps to chromosome 1, between CAPS marker GAPB and SSLP marker nF19K23. Genetic analysis indicates that the transposon in this line and the *scz* mutation are unlinked.

There are no alternating files of root hair and non-root hair cells in the epidermis of *scz* mutants (Fig. 1B) as there are in wild type (Fig. 1A). In addition, some of the hairs emerged from cells in the subepidermal location and grew out between the cells of the epidermis (arrows in Fig. 1B).

Root hairs are formed from the subepidermal layer of *scz* mutant roots

Fig. 2A is a median longitudinal section through a 3-day-old root of a plant homozygous for the *scz* mutation. The image shows two root hair cells. Transverse sections through the root, at positions indicated by arrows, show a root hair that originates from a cell in the subepidermal location (asterisks in Fig. 2B). In 3-day-old plants approximately 60% of the hairs originate from the layer immediately underneath the epidermis

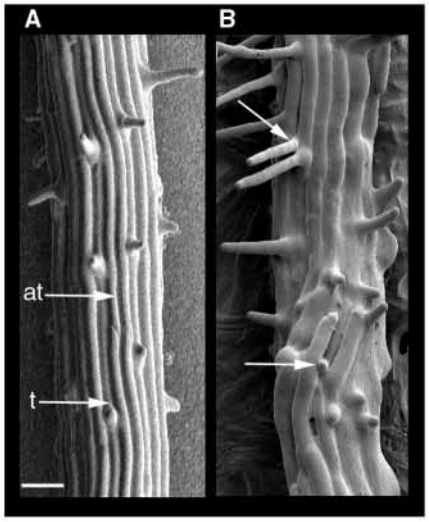

Fig. 1. Morphology of 3-day-old *scz* root. Scanning electron micrograph of the root at the beginning of the differentiation zone. The tubular outgrowths are root hairs. (A) The alternating files of trichoblasts (t) and atrichoblasts (at) can be seen in the wild-type Landsberg *erecta*. (B) There are no alternating files of trichoblasts and atrichoblasts in *scz* mutants. Furthermore, root hairs can be seen originating from the underlying cells (arrows). Bar, 50 μm.

(subepidermal layer) but not every cell in this layer produces a hair. By 10 days, almost all cells in the subepidermal layer have developed hairs.

To determine if subepidermal cells display other epidermis-specific traits, we examined the expression of the GFP-enhancer trap line J0481 in the root hair region of *scz* mutant roots. This enhancer trap is expressed in all the epidermal cells of the elongation and differentiation zones in wild-type roots (Fig. 2D). In contrast the enhancer trap line is expressed in the epidermis and the underlying layers of 3- to 7-day-old *scz* roots (Fig. 2C) and every subepidermal root hair-forming cell expresses the marker. The *GL2::GUS* promoter fusion is preferentially expressed in epidermal atrichoblasts (cells that will develop into non hair-bearing epidermal cells) in wild type (Masucci et al., 1996). *GL2::GUS* is expressed in some subepidermal cells in the *scz* mutant (data not shown), further supporting the notion that the subepidermal layer exhibits epidermal traits. We examined the expression of a ground tissue-specific marker (J0571) and two endodermis-specific markers (J3611 and J2672) in the *scz* mutants. These enhancer traps are not expressed in the hair-forming subepidermal cells of *scz* mutants (data not shown), suggesting that the cells display epidermal but not ground tissue traits.

Epidermal patterning genes act in the hair-forming subepidermal layer of *scz* mutants

TRANSPARENT TESTA GLABRA (*TTG*), and *GLABRA2* (*GL2*) repress hair cell identity in the epidermis (Galway et al., 1994; Masucci et al., 1996; Di Cristina et al., 1996). *CAPRICE* (*CPC*), in contrast, is a positive regulator of hair formation in the epidermis (Wada et al., 1997). To determine if *TTG*, *GL2* and *CPC* are active in the *scz* subepidermal layer, double mutants with *ttg*, *gl2* and *cpc* were generated. The phenotypes of *scz*, *scz gl2*, *scz ttg* and *scz cpc* mutants are shown in Fig. 3. *scz gl2* and *scz ttg* double mutants (Fig. 3B,C upper panels,

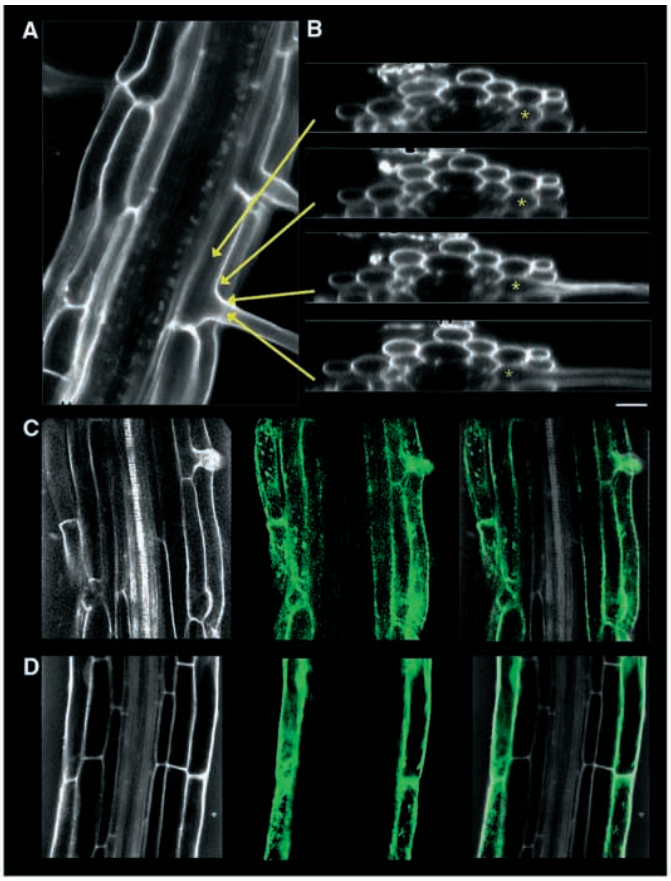

Fig. 2. The subepidermal layer of *scz* mutants develops root hairs and expresses epidermal cell specific markers. (A) Median longitudinal optical section through the *scz* mutant root showing two cells with root hairs arising from subepidermal cell layers. (B) Transverse sections through the root shown in A at positions indicated by arrows illustrate that the root hair originates from the cortex (asterisk). (C,D) Expression of GFP-enhancer trap line J0481 in the differentiation zone of a *scz* mutant root (C) and wild-type root (D). Median longitudinal sections stained with propidium iodide are shown on the left, GFP expression (green) is in the middle and superimposed images are shown on the right. The enhancer trap is expressed in the epidermis and ground tissue in *scz* mutants (C), in contrast to wild type where expression is restricted to the epidermis (D). Bar, 25 μm.

respectively) have more root hairs than *scz* single mutant (Fig. 3A upper panel). In both double mutants almost all epidermal cells form root hairs. In addition, most if not all cells situated in a subepidermal position form root hairs, as shown in 3-day-old *scz gl2* and *scz ttg* roots (Fig. 3B,C lower panels, respectively), compared with *scz* mutants where fewer cells of the subepidermal layer form root hairs (Fig. 3A lower panel). *scz cpc* double mutants develop fewer root hairs than *scz* single mutants (compare Fig. 3D to A upper panel). Of the few root hairs that form in the *scz cpc* double mutant, some originate from subepidermal cells (Fig. 3D lower panel).

In conclusion, *ttg* and *gl2* enhance root hair formation in the subepidermal layer of *scz*, while *cpc* decreases the number of hairs. This indicates that *TTG*, *GL2* and *CPC* act in the subepidermal layer of *scz* in the same way as they act in the epidermis of the *scz* mutant and the wild type, supporting the

view that the subepidermal layer of the *scz* mutant has epidermal characteristics.

SCZ is required during cell division in the meristem

Meristems of 3-day-old *scz* primary roots were analysed and compared to wild-type (L*er*) meristems. The cellular organisation of the *scz* mutant meristem is defective – the planes of division are abnormal compared to wild type and the organisation of the cells next to the presumptive quiescent centre (initial cells) is altered. Consequently it is often difficult to precisely define the position of the epidermis/lateral root cap initials and daughter cells (asterisks, Fig. 4C). During the development of the ground tissue in wild type the cortex/endodermis initial divides anticlinally to generate a new initial and a daughter cell. This daughter in turn undergoes an asymmetric periclinal division to generate an outer cell, which gives rise to cortical cells, and an inner cell, which gives rise to the file of endodermal cells (arrowhead, Fig. 4B). In *scz* mutants a number of anticlinal divisions may take place before the periclinal division occurs (arrowhead, Fig. 4C). More than one periclinal division may occur in any lineage, here defined as the descendants of a single cortical endodermal initial, which results in an increase in the number of cell layers (arrows, Fig. 4C).

An epidermis-specific enhancer trap is expressed in the ground tissue of *scz* mutant meristem

To determine if the mis-specification of the ground tissue (cortex/endodermis) occurs during early development, in the meristem, the expression of the GFP enhancer trap line N9173 was examined in *scz* mutant roots. In wild type, this enhancer trap is expressed after division of the daughter cells of the lateral root cap and the epidermal initial (arrow, Fig. 5A) and expression is maintained in all the cells of the epidermis and the lateral root cap (Fig. 5A). In *scz* roots, the enhancer trap is expressed in these cell types but is also expressed at low levels in cells of the extra layer that forms as a result of the supernumerary periclinal division in the ground tissue. The expression is weaker than in the epidermis/lateral root cap and it occurs in clusters of cells (arrowheads, Fig. 5B,C). This suggests that the subepidermal layers of the *scz* roots exhibit epidermal characteristics in the meristem as well as in the mature zone where hairs develop (as shown above). Furthermore the expression of the enhancer trap is displaced upwards (away from the root tip) in *scz* mutants compared to wild type (arrow, Fig. 5B). This suggests that *SCZ*

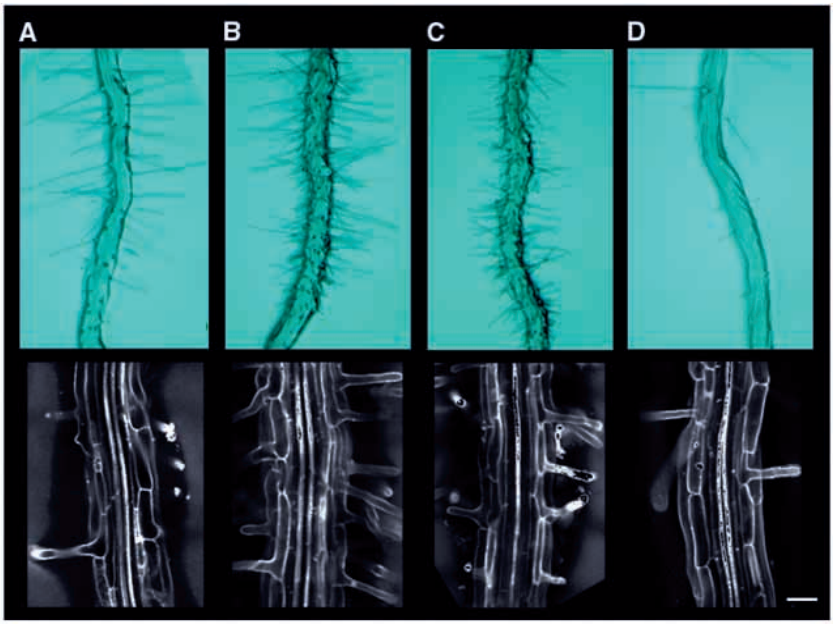

Fig. 3. *TTG*, *GL2* and *CPC* act in the subepidermal layer of *scz* mutants. (A) *scz*; (B) *scz gl2*; (C) *scz ttg* and (D) *scz cpc*. (Upper panels) Light micrographs of the beginning of the differentiation zone. *scz gl2* and *scz ttg* form more root hairs than *scz*, while *scz cpc* form fewer root hairs. (Lower panels) Median longitudinal sections through the beginning of differentiation zone, showing that the number of subepidermal root hairs is altered in the double mutants when compared to *scz* single mutant. Bar, 50 μm.

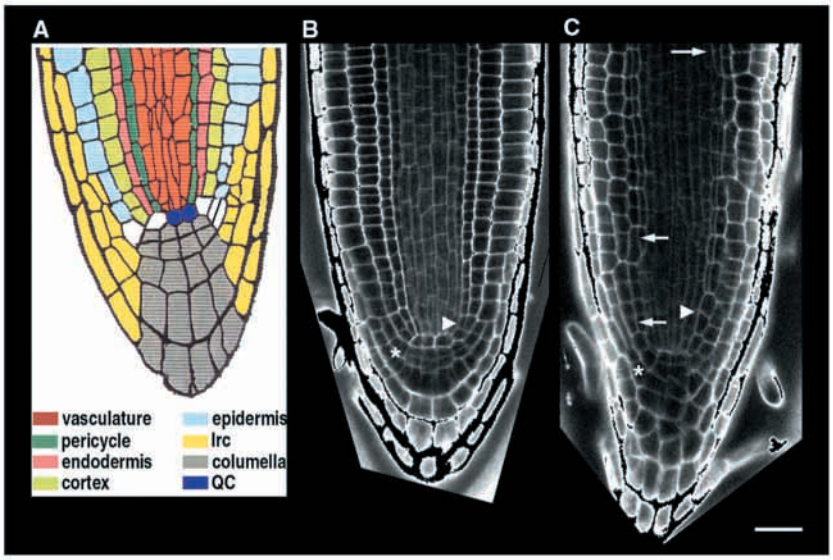

Fig. 4. Defects in the cellular organisation of *scz* meristem. (A) Schematic representation of meristem organisation of the *Arabidopsis* root. The colour code identifies the different cell types. The initials of the epidermis/lateral root cap and cortex/endodermis are shown in white. (B,C) Median longitudinal section through 3-day-old wild-type (B) and *scz* (C) roots. (B) In wild type, the asterisk indicates a cell that has divided asymmetrically to generate epidermal (inner) and lateral root cap (outer) daughter cells and the arrowhead indicates a cell that has divided asymmetrically to produce the endodermis (inner) and cortex (outer) daughter cells. (C) In *scz*, the epidermis/lateral root cap daughter cells are not clearly defined (asterisk). Division pattern of the cortex/endodermis daughter cell is abnormal. Often an anticlinal division occurs instead of a periclinal one (arrowhead). More than one periclinal division can occur (arrows) resulting in supernumerary layers (C). Bar, 25 μm.

may be required for position-dependent development along the apical basal axis of the root.

A ground tissue-specific enhancer trap is mis-expressed in *scz* mutant meristems

Since an epidermis-specific enhancer trap is mis-expressed in the meristem of the *scz* roots, we predicted that the expression of ground tissue-specific genes would be defective in the subepidermal layers of *scz* roots. In wild type, the J0571 enhancer trap is expressed in the quiescent centre, the cortex/endodermis initials, cortex/endodermis daughter cells and throughout the cortex and endodermis (Fig. 6A). The expression pattern of this enhancer trap is altered in the *scz* meristem. In regions of the mutant root where additional ground tissue layers develop the enhancer trap is expressed in the two layers of the ground tissue next to the vascular cylinder. Furthermore the enhancer trap is not expressed in the presumptive quiescent centre, or in the ground tissue initials. Along the apical basal axis the expression is first observed after the first periclinal divisions have taken place (asterisk, Fig. 6B), i.e. it is displaced upwards along the root as is the case for the epidermal enhancer trap (N9173). Expression in the meristem is discontinuous (arrow, Fig. 6B). Occasionally, there are clusters of a few cells in the extra ground tissue layer, below the epidermis, that express the enhancer trap (arrowhead, Fig. 6B). Hence, very few cells of the supernumerary ground tissue layers express the ground tissue specific marker. This is consistent with the majority of the cells in the subepidermal layer being epidermal in identity. The mis-specification of the subepidermal layer as epidermis is mostly evident in the elongation/differentiation zone.

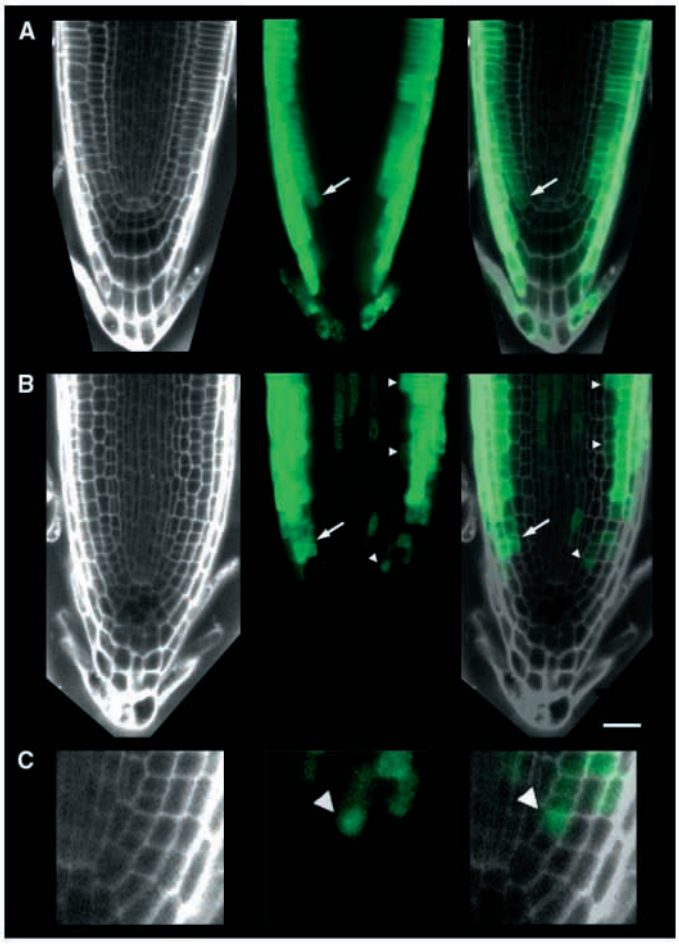

Fig. 5. An epidermis-specific enhancer trap is expressed in cells of the ground tissue in *scz* meristems. Expression of GFP-enhancer trap line N9173 in 3-day-old wild-type (A) and *scz* (B) meristems. (A) In wild type, the enhancer trap is expressed in the lateral root cap and the epidermis. Expression is evident immediately after division of the epidermis and lateral root cap daughter cells (arrow). (B) In *scz* mutants, expression in the epidermis starts higher in the root (arrow). Moreover, in the ground tissue clusters of cells show a lower level of expression (arrowheads). (C) Close-up of a cluster of cells shown in B illustrating expression of the marker in the ground tissue. Bar, 25 μm in A,B; 56 μm in C.

scz interacts with *scr* and *shr*

SCARECROW (*SCR*) and *SHORT ROOT* (*SHR*) are required for the development of the ground tissue and *scr* and *shr* mutant roots develop a single layer of ground tissue instead of two (Benfey et al., 1993; Scheres et al., 1995). To determine if *SCZ* is involved in the same process as *SHR* and *SCR*, double mutant plants for *scz* and either *scr* or *shr* were constructed. Both *scz scr* and *scz shr* have fewer cell layers than the *scz* single mutant (Fig. 7A,B respectively), indicating that *scr* and *shr* suppress the extra periclinal divisions occurring in the *scz* ground tissue. Occasionally, periclinal divisions are observed in *scz scr* ground tissue (arrow, Fig. 7A). Such divisions lead to the formation of a partial double ground layer as has been already reported in the *scr* single mutant (Wysocka-Diller et al., 2000).

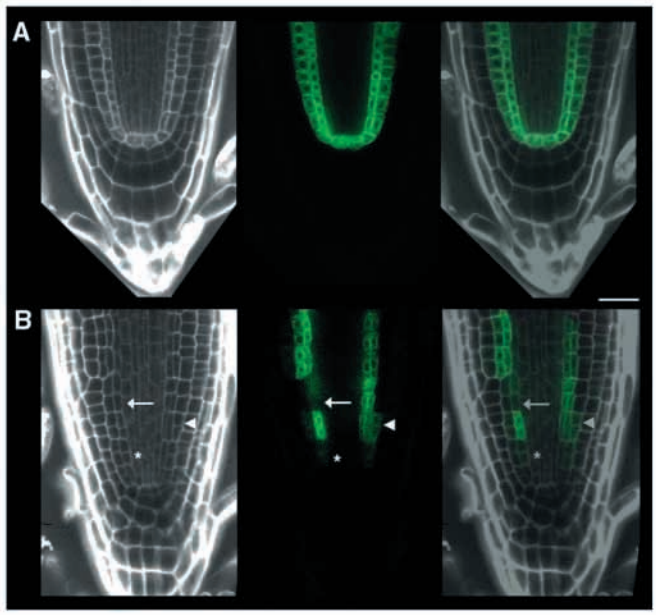

Fig. 6. The expression of a cortex/endodermis-specific enhancer trap is altered in the *scz* meristem. Expression of the GFP-enhancer trap line J0571 in 3-day-old wild-type (A) and *scz* (B) meristems. (A) In wild type the marker is expressed in the quiescent centre, the cortex/endodermis initial, the cortex/endodermis daughter cells and in all the cells of the cortex and the endodermis. (B) The expression of the marker is displaced upwards (asterisk) in *scz* mutants. The expression is discontinuous (arrow). There are clusters of cells in the extra outer ground tissue layer that show low levels of expression (arrowhead). Bar, 25 μm.

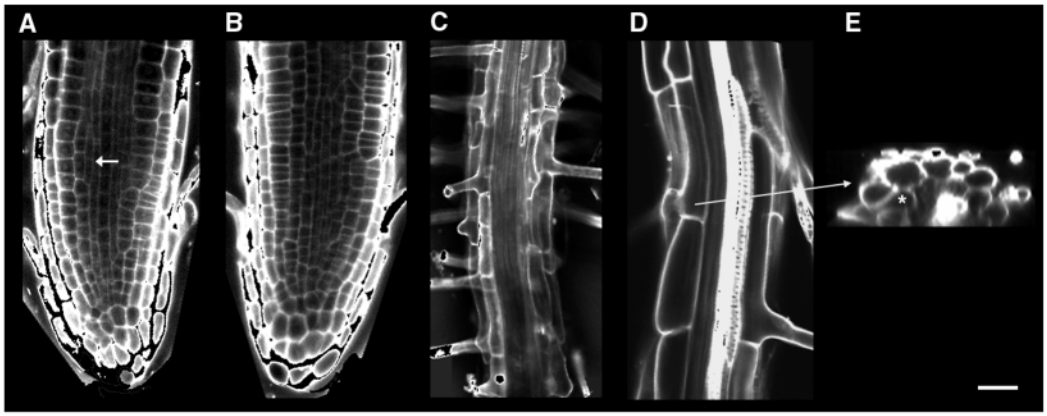

Fig. 7. *scz* interacts with *scr* and *shr*. (A,B) Median longitudinal optical sections through the meristem of 3-day-old *scz scr* (A) and *scz shr* (B) double mutant roots. Both *scr* and *shr* suppress periclinal divisions in the ground tissue of *scz* mutants. Occasionally periclinal divisions occur in the ground tissue of *scz scr* (arrow). (C,D) Median longitudinal section in the differentiation zone of 3-day-old *scz scr* (C) and *scz shr* (D) double mutant roots. Cells of the ground tissue in the *scz scr* double mutant form root hairs (C). Few of the ground tissue cells in the *scz shr* double mutants form root hairs (D), as shown in an optical cross section (E, asterisk). Bar, 25 μm in A,B,D,E; 50 μm in C.

scz scr and *scz shr* double mutants have a single layered epidermis and ground tissue, and the ground tissue forms root hairs (Fig. 7C,D and E respectively) indicating that *scr* and *shr* do not suppress subepidermal hair cell development. This suggests that the supernumerary periclinal cell divisions are not required for the mis-specification of cells in the ground tissue.

In conclusion, both *scr* and *shr* suppress the extra periclinal divisions that take place in *scz* mutants indicating that these genes may act in the same pathway. Nevertheless root hairs are formed in the single cell layer ground tissue of the double mutants, indicating that the mis-specification of the ground tissue of *scz* mutants is not a consequence of the formation of extra ground tissue layers.

SCR expression in *scz* mutants

SCR is required for the execution of the periclinal cell division of the cortex/endodermis initial daughter cell, generating the cortex and endodermis cell files (Di Laurenzio et al., 1996). Since *shr* and *scr* suppress the development of additional cell layers in *scz* mutants, it is possible that *SCZ* regulates the transcription of *SCR* directly and that the extra periclinal divisions occurring in *scz* mutants are the result of the deregulation of *SCR* expression. To test this hypothesis mRNA was isolated from roots of 3-day-old *scz* and wild-type plants. The amount of *SCR* mRNA was determined by semi-quantitative RT-PCR. As it is shown in Fig. 8 (upper panel), there are the same amounts *SCR* mRNA in *scz* mutants and in wild type. As a control for equal amounts of cDNA used for amplification (Fig. 8 lower panel), we used the *Arabidopsis* elongation factor 1-alpha 4 (Liboz et al., 1989). Since *scz* mutation does not affect the steady state levels of *SCR* mRNA, it is unlikely that *SCZ* regulates *SCR* transcription.

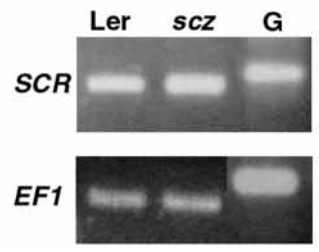

Fig. 8. Expression of *SCR* in *scz*. RT-PRC was performed to compare the amounts of *SCR* mRNA in *scz* mutant and wild-type L*er* roots (upper panel). The *Arabidopsis* elongation factor 1-alpha 4 (*EF1*) was used as a standard (lower panel). Lane G shows the size of the fragment synthesised when genomic DNA is used as a template.

Defects in divisions are first seen in embryos

To determine when *SCZ* is first active during the life of the plant, the organisation of cells in *scz* embryos was compared to wild type at different developmental stages. At the early heart stage *scz* and wild-type embryos were indistinguishable – cell layers and initials were laid down around the quiescent centre as in wild-type embryos (data not shown). Differences in cell division patterns between wild type and *scz* mutant were first seen during the transition from heart to torpedo stage when the promeristem is formed (Fig. 9A). The periclinal division of the presumptive cortex/endodermis initial daughter cell takes place earlier in the *scz* mutant than in wild type (arrow, Fig. 9A). This suggests that *SCZ* activity is required between the heart and the torpedo stages of embryogenesis.

At the walking stick stage in *scz* mutant embryos the pattern of cell divisions in the ground tissue is altered. A periclinal division occurs at one cell distance from the presumptive central cells (arrowhead, Fig. 9B) resulting in the formation of two files. A second periclinal division takes place in the cells of the inner file. These combined divisions result in the formation of three ground tissue cell layers in the *scz* embryo instead of the two that normally develop in wild type (cortex and endodermis). The ectopic periclinal divisions resulting in the formation of an extra cell layer were observed in all *scz* mutant embryos examined (n=8). These data indicate that the control of the position and number of periclinal divisions of the cortex/endodermis initial daughter cell is defective in *scz* mutants. This results in the formation of supernumerary cell layers in developing embryos.

DISCUSSION

We have shown that *SCZ* is required for the establishment of the radial organisation of tissues in the root. The development of subepidermal root hair cells in the *scz* mutant indicates that *SCZ* represses epidermal identity in the ground tissue of wild-

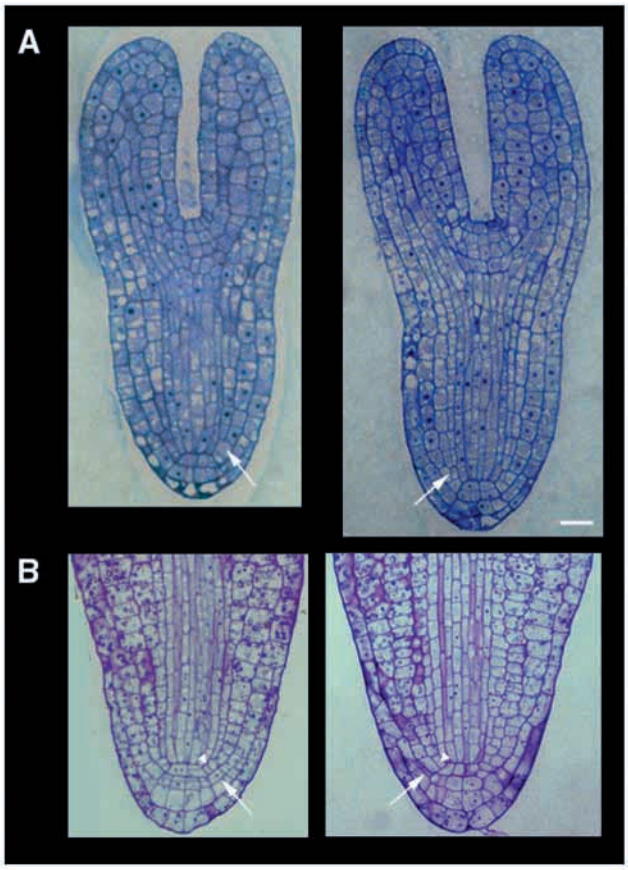

Fig. 9. Cell division defects in *scz* embryos. Wild-type (left panels) and *scz* mutant (right panels) embryos stained with Toluidine Blue. (A) In torpedo stage *scz* embryos, a periclinal division of the presumptive cortex/endodermis initial daughter cell occurs earlier (arrow) than in wild type. (B) In walking stick embryos, an extra periclinal division in *scz* mutants results in the formation of a new layer in the ground tissue (arrowhead). The periclinal division of the epidermis/lateral root cap initial is indicated with an arrow. Bar, 25 μm.

type roots. Furthermore the development of additional layers in the ground tissue of the *scz* mutant root indicates that *SCZ* also functions to repress periclinal divisions in the ground tissue of wild-type roots. Taken together these data suggest that *SCZ* plays a pivotal role in the development and maintenance of ground tissue in the root.

SCZ is required for suppression of epidermal fate in the ground tissue

Evidence presented here indicates that *SCZ* is required to repress epidermal identity in subepidermal layers of the root, i.e. to restrict epidermal fate to the outer layer of the mature root. The subepidermal layer in *scz* mutants is transformed into an epidermis as revealed by the formation of root hairs and the expression of molecular markers in these cells. We examined the behaviour of 3 independent GFP enhancer trap lines expressed in the epidermis in the *scz* background (J0481, J0672 and J2092). All lines showed expression in the subepidermal layer(s). In addition, the *GL2* gene, which is normally only expressed in the atrichoblasts and non hair cells of the epidermis, is expressed in cells of the subepidermal layer of the *scz* mutant. Further support that the *scz* subepidermal layer displays characteristics of epidermis comes from double mutants in which *scz* is combined with other mutations affecting the development of the epidermis. The *TTG*, *GL2* and *CPC* genes active in the specification of cell fate in the epidermis are also active in the subepidermal layer of *scz* mutant roots, supporting the view that the subepidermal layer in *scz* mutants is epidermal in character. While the subepidermal cells of *scz* roots are located where cortical cells are situated in a wild-type root, we found no evidence, using molecular markers, that these cells exhibit cortical identity (J0571). One possibility is that *SCZ* represses the expression of genes required for epidermal identity and promotes the expression of those required for cortical cell development in the ground tissue. In addition, some of the markers expressed in cortex or endodermis used for the analysis showed no expression in the mutant background, indicating that the genes are downstream of the *SCZ* pathway (J2931, Q2393, J3611 and J2672). Alternatively the lack of expression might be a result of ground tissue mis-specification.

The development of epidermal trichomes in subepidermal tissues has been described in leaves that constitutively express *GLABRA1 (GL1)* in a *triptyphon* (*try*) mutant background (Schnittger et al., 1998; Szymanski and Marks, 1998). Since *GL1* is a positive regulator of trichome development, its ubiquitous expression might be expected to predispose cells to trichome development. When misexpressed in the absence of *TRY*, the negative regulator of trichome development, cells in subepidermal tissues then differentiate as trichomes. Given the dominant, gain-of-function effect of overexpressing *GL1* this observation does not indicate that either *GL1* or *TRY* are active in repressing or promoting trichome development in the subepidermal tissues of leaves during normal development. Rather it suggests that the fate of cells can be switched if the appropriate regulatory genes are expressed or repressed. On the contrary our observation that plants homozygous for *scz* presumptive loss-of-function mutations develop root hairs from the subepidermal layers suggests that *SCZ* is required to repress epidermal cell differentiation in internal cells.

SCZ represses periclinal divisions in the ground tissue

Plants homozygous for the presumptive loss of function *scz* mutation develop supernumerary cell layers because of an increased number of periclinal divisions in cells of the ground tissue. Such deviations from the wild-type pattern occur during embryogenesis when the cellular organisation of the future root meristem is being laid down. Defects are also observed during the formation of lateral root meristems, indicating that *SCZ* is required for the establishment of radial pattern in roots throughout the life of the plant.

In wild type, the cortex/endodermis initial daughter cell undergoes a single periclinal division giving rise to cortical and endodermal daughter cells. Anticlinal divisions in each of these cells result in the formation of groups of cortical and endodermal cells (Fig. 10A). In *scz*, additional rounds of periclinal cell divisions occur, resulting in the formation of a multi-layered ground tissue. We propose that in *scz* a periclinal division results in the formation of two cells. The outer cell continues to divide anticlinally generating a new layer. The inner cell may then undergo an additional periclinal division resulting in the formation of two files of cells. This process can

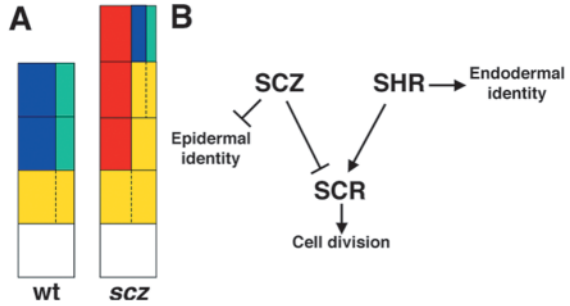

Fig. 10. (A) Schematic representation of cell divisions and the resulting ground tissue lineage in wild type (wt) and *scz*. The periclinal divisions are shown with a dotted line. The cortex/endodermis initial is shown in white, the cortex/endodermis initial daughter cell in yellow, the cortex in blue, the endodermis in green and the extra cell layer of *scz* in red. (B) Model of interaction of *SCZ*, *SCR* and *SHR*. See text for a detailed discussion.

be repeated a number of times resulting in the formation of a multi-layered ground tissue (Fig. 10A).

In addition to the cell division defects of *scz*, the expression of the enhancer traps is also altered, indicating that *SCZ* may be involved in organising the tissues of the root. A number of enhancer traps, that are expressed in cells in the vicinity of the quiescent centre in wild type, are displaced upwards. This suggests that the *scz* mutant displays a defect along the apical-basal axis similar to that described for roots exposed to auxin transport inhibitors for extended periods of time (Sabatini et al., 1999). Furthermore, the defective organisation of cells in the root meristem in mutants with either defective auxin responses or auxin transport suggests that perception and transport of auxin are involved in patterning (Sabatini et al., 1999; Friml et al., 2002). The precise role of auxin in the cell division and cell specification defects of *scz* remains to be tested.

Periclinal division in the cortex/endodermis daughter cells does not occur in *shr* mutants, while ectopic expression of *SHR* results in extra periclinal divisions and the consequent development of supernumerary layers (Helariutta et al., 2000). Since the pattern of cell divisions in *scz* roots resembles those found in plants that overexpress *SHR*, it is likely that *SCZ* and *SHR* play opposite roles: *SHR* is a positive regulator of the asymmetric cell division and *SCZ* is a negative regulator. That these genes may act in the same pathway is supported by the observation that the *scr* and *shr* mutations suppress the Scz- phenotype of *scz* mutants.

SHR has been shown to act as a transcriptional activator of *SCR*. Our data suggest that it is unlikely that *SCZ* is a transcriptional repressor of *SCR*, indicating that *SCZ* represses SCR activity. A plausible explanation for *SCZ* action in the ground tissue is that it represses SCR cell division activity in the inner daughter cell after the periclinal division of the cortex/endodermis initial daughter cell. The existence of such a repressor is in accordance with the fact that expression of *SCR* in the endodermis daughter cell and in the endodermal cells does not result in continuous rounds of cell division (Fig. 10B). While the extra periclinal divisions of *scz* roots are suppressed by *shr* and *scr*, the defective development of hair cells from subepidermal layers is not suppressed in *scz scr* and *scz shr* double mutants. This indicates that specification of the ground tissue is independent of the initial cell division pattern.

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