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Report

SCHIZORIZA Encodes a Nuclear Factor Regulating Asymmetry of Stem Cell Divisions in the *Arabidopsis* Root

Colette A. ten Hove,[1] Viola Willemsen,[1] Wouter J. de Vries,[1] Anja van Dijken,[1] Ben Scheres,[1] and Renze Heidstra[1,*]
[1]Molecular Genetics, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Summary

Cell divisions generating daughter cells different in size, shape, identity, and function are indispensable for many developmental processes including fate specification, tissue patterning, and self-renewal. In animals and yeast, perturbations in factors required for well-described asymmetric cell divisions generally yield cells of equal fate. Here we report on SCHIZORIZA (SCZ), a single nuclear factor with homology to heat-shock transcription factors that controls the separation of cell fate in a set of stem cells generating different root tissues: root cap, epidermis, cortex, and endodermis. Loss-of-function, expression, and reconstitution experiments indicate that SCZ acts mainly from within its cortical expression domain in the stem cell niche, exerting both autonomous and nonautonomous effects to specify cortex identity and control the separation of cell fates in surrounding layers. Thus, SCZ defines a novel pathway for asymmetric cell division in plants.

Results and Discussion

Asymmetric cell division is a fundamental and universal mechanism for generating diversity and pattern in multicellular organisms [1, 2]. The radial organization of the *Arabidopsis* root is derived from stereotyped asymmetric cell divisions of different stem cells, the initials. These cells and their daughters produce defined tissue layers with distinct cell fates (Figure 1A) [3]. The stem cells surround a small group of rarely dividing cells, the quiescent center (QC), required for their maintenance. The QC itself is formed early during embryogenesis when an asymmetric division of the hypophyseal cell forms the lens-shaped QC progenitor cell and future columella root cap [4]. QC fate is specified in parallel by the PLETHORA (PLT), SHORT ROOT (SHR), and SCARECROW (SCR) transcription factors [5–7]. SHR and SCR are also required for ground tissue patterning: *shr* and *scr* mutants lack the asymmetric periclinal division in the ground tissue stem cell daughter, resulting in a single ground tissue layer. For *shr*, this layer lacks endodermal identity, but in *scr*, this layer displays mixed cortical/endodermal identity [8–10]. Several other reports have appeared that suggest plant cells may possess mixed fates [11–13]. To our knowledge, the only known example of mixed fate phenotypes in animal development comes from extensive genetic screening approaches in *C. elegans*, which have uncovered mutants in which a single neuronal fate decision is inappropriately executed, resulting in a mixed fate phenotype [14]. Here, we describe the SCHIZORIZA (SCZ) nuclear factor, which is required for plant cell fate separation in several tissues, acting both cell-autonomously and non-cell-autonomously. Our data highlight a novel mechanism of cell fate separation in plants that is particularly relevant for asymmetric cell divisions within stem cell areas.

SCZ Encodes a Member of the Heat-Shock Transcription Factor Family

To find novel genes involved in QC specification and stem cell maintenance, we performed a QC marker-based mutagenesis screen. A line doubly homozygous for *QC25* and *QC46* promoters [7] fused to $_{ER}$*CFP* and $_{ER}$*YFP*, respectively (Figures 1B and 1C), was mutagenized, and the M2 progeny were analyzed for altered expression. Five phenotypically indistinguishable mutant lines combined reduced *QC25::*$_{ER}$*CFP* and *QC46::*$_{ER}$*YFP* activity with retarded root growth and disorganization of the stem cell niche (Figures 1D and 1E; see also below). Complementation tests showed that all five lines carried allelic mutations. Given the equal allele strength, we continued to use one allele, *qc351*, for further analysis.

Compared to wild-type, *qc351* roots developed more hairs, lacked the stereotypical pattern of alternating hair and nonhair files, and initiated root hairs from subepidermal tissue reminiscent of the *schizoriza* (*scz*) mutant phenotype (see Figures S1A and S1B available online) [15]. Complementation analysis revealed that *qc351* was allelic to *scz*. Accordingly, we renamed our mutant alleles *scz-2* through *scz-6*.

We molecularly characterized the *scz* mutation via a map-based approach. Fine mapping located *SCZ* to a single locus in an area of 70 kb on chromosome 1 (Figure 1G). One of the candidate genes that we sequenced was the *heat shock transcription factor B4* (*HsfB4*) locus (At1g46264), based on its stem cell-enriched in silico root expression pattern (http://www.arexdb.org/; [16–18]. Comparison with the corresponding wild-type sequence revealed different mutations in the *HsfB4* gene for all *scz* alleles, including two additional TILLING alleles (Figure 1G; Table S1). The identical phenotype indicates that they are likely *HsfB4* mutant null alleles. For *Arabidopsis*, the 21 Hsfs are classified into three major groups, A (16 members), B (4 members, including *SCZ*), and C (1 member), according to the different flexible linkers in their HR-A/B oligomerization regions (Figure 1G, green bar). Despite considerable diversification in size and sequence, the basic structure of Hsfs is conserved among eukaryotes [19].

SCZ mRNA is first detected at triangular-stage embryos in the QC progenitor cells. From heart stage onward, *SCZ* mRNA accumulation expands into ground and vascular tissue progenitors and their immediate daughters (Figures 2A–2C). This expression pattern is maintained in the postembryonic root with highest *SCZ* mRNA accumulation in QC and ground tissue stem cells and their immediate daughters (Figure 2D). In the *scz-2* mutant, hybridization signal is absent from the subepidermal layer (Figures S2A and S2B). *SCZ* promoter-reporter fusions essentially corroborate the in situ hybridization expression pattern (Figures S2C–S2E). Consistent with its function as a putative transcription factor, the complementing 35S::GFP:SCZ translational fusion localizes to the cell nucleus (see below).

*Correspondence: r.heidstra@uu.nl

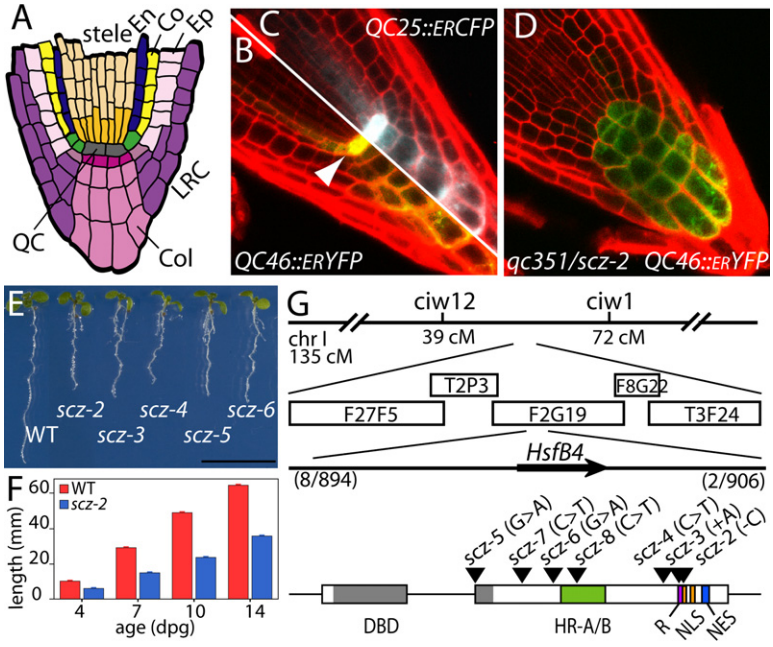

Figure 1. Identification and Cloning of *SCZ*

(A) Schematic view of the *Arabidopsis* root meristem. The following abbreviations are used: En, endodermis; Co, cortex; Ep, epidermis; QC, quiescent center; Col, columella; LRC, lateral root cap.

(B–D) Four-day-old wild-type root expressing both *QC46::$_{ER}$YFP* (B; arrowhead indicates QC) and *QC25::$_{ER}$CFP* (C) and 4-day-old *qc351/scz-2* root showing *QC46::$_{ER}$YFP* expression (D; false green).

(E) Eight-day-old wild-type and *scz-2* through *scz-6* seedlings.

(F) Root length (in mm) of wild-type and *scz-2* seedlings at the indicated number of days postgermination (dpg). For each data point, n $\geq$ 57; error bars indicate standard error of the mean.

(G) Schematic representation of the identification of the *SCZ* gene by positional cloning. *SCZ*/*HsfB4* location is shown relative to a contig of five BAC clones; the proportion of recombinant seedlings is shown in parentheses. Boxes indicate coding sequence. Conserved functional domains according to [19] are indicated as follows: gray, conserved DNA-binding domain; green, HR-A/B oligomerization region; orange, nuclear localization signal (NLS); blue, nuclear export signal (NES); purple, R (R/KLFGV) motif [33]. The positions of the nucleotide sequence changes are shown for each mutant allele; *scz-7* and *scz-8* are TILLING alleles [34]. See also Figure S1.

Disturbed Asymmetric Cell Division from Embryogenesis Onward in *scz*

The reduction in root growth together with altered expression of the QC markers *QC25::$_{ER}$CFP* and *QC46::$_{ER}$YFP* in mutants (Figures 1D and 1E) might indicate a role for *SCZ* in QC/stem cell specification and/or function. However, *scz-2* roots continue to grow in an indeterminate manner, with root length lagging by about half behind wild-type (Figure 1F). Similarly, root meristem size is reduced but maintained in *scz-2* (Figure S1C). These observations show that *SCZ* is not critically required for stem cell maintenance.

Cells at the position of the QC and columella stem cells in *scz-2* mutant roots are morphologically abnormal and accumulate starch granules, which marks differentiated columella in wild-type (Figures 2F and 2G). QC25, QC46, and QC184 markers are displaced from the QC and express diffuse activity in the starch granule-containing cells. *WOX5* also marks the QC, and the gene product is required to maintain the underlying columella stem cells. *WOX5::$_{ER}$GFP* expression faded from the position of the QC but did not appear in the columella (Figures 2F–2I; Figures S2F–S2I). Apparently, QC and columella fates are present but not separated in *scz-2* roots, and

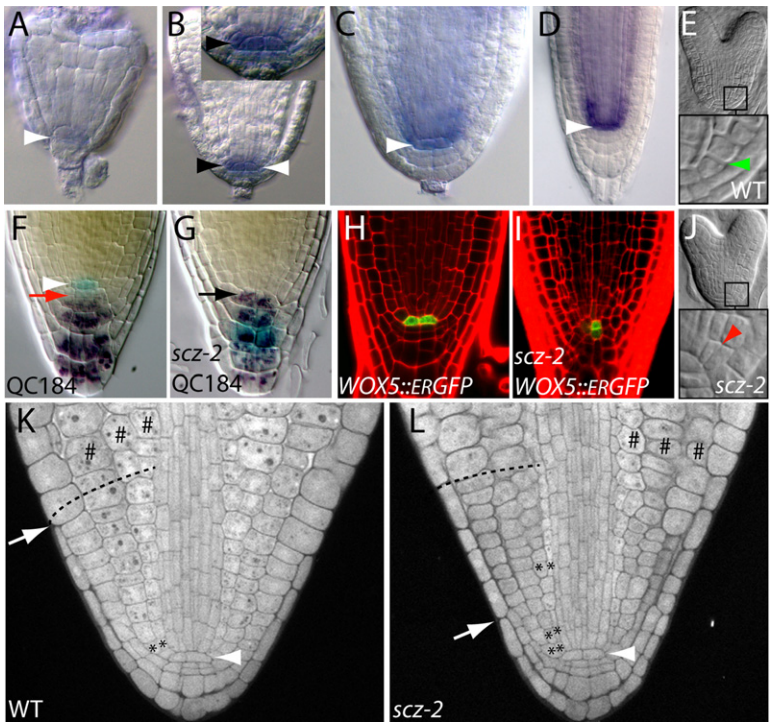

Figure 2. *SCZ* Expression and Postembryonic Mutant Stem Cell Defects

(A–D) In situ hybridization with *SCZ* antisense probe in wild-type triangular- (A), heart- (B; black arrowhead marks ground tissue accumulation), and torpedo-stage (C) embryo and 2-day-old seedling (D). White arrowheads in (A)–(D), (F), (K), and (L) indicate QC.

(E and J) Heart-stage wild-type (WT; E) and *scz-2* (J) embryos. Inset shows magnification of normal anticlinally divided ground tissue initial (E; green arrowhead) and aberrant periclinally divided ground tissue initial (J; red arrowhead).

(F and G) *QC184* expression (blue) in 6-day-old wild-type (F) shifts in the columella in *scz-2* (G) roots. Red arrow indicates columella stem cells devoid of purple starch granules; black arrow indicates accumulation of starch granules in mutant QC region.

(H and I) *WOX5::$_{ER}$GFP* expression in 4-day-old wild-type (H) and *scz-2* (I) roots.

(K and L) Aniline blue-stained wild-type (K) and *scz-2* (L) mature embryos. White arrow indicates end of lateral root cap layer, dashed line indicates root/hypocotyl boundary, # indicates ground tissue layers in hypocotyl, * indicates periclinal ground tissue divisions leading to supernumerary mutant layers in *scz-2*. See also Figure S2.