Developmental Biology 365 (2012) 14–22

Contents lists available at SciVerse ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Review

Surfing along the root ground tissue gene network

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ARTICLE INFO

Article history:
Received for publication 30 September 2011
Revised 31 January 2012
Accepted 6 February 2012
Available online 12 February 2012

Keywords:
Ground tissues
Root apical meristem
Endodermis
Cortex
Arabidopsis thaliana
Rice
SHORT-ROOT
SCARECROW
SCHIZORHIZA

ABSTRACT

Organization of tissues in *Arabidopsis thaliana* root is made of, from outside in, epidermis, cortex, middle cortex, endodermis, pericycle and vascular tissues. Cortex, middle cortex and endodermis form the ground tissue (GT) system. Functional and molecular characterization of GT patterning mutants' properties has greatly increased our understanding of fundamental processes of plant root development. These studies have demonstrated GT is an elegant model that can be used to study how different cell types and cell fates are specified. This review analyzes GT mutants to provide a detailed account of the molecular network that regulates GT formation in *A. thaliana*. The most recent results indicate an unexpectedly complex network of transcription factors, epigenetic and hormonal controls that play crucial roles in GT development. Major differences exist between GT formation in dicots and monocots, particularly in the model plant rice, opening the way for evo–devo of GT formation in angiosperm. In rice, adaptation to submergence relies on a multilayered cortex. Moreover, variation in the number of cortex cell layers is also observed between the five root types. A mechanism of control for cortical cell number should then exist in rice and it remains to be determined if any of the *Arabidopsis thaliana* identified GT network members are also involved in this process in rice. Alternatively, a totally different network may have been invented. However, first available results suggest functional conservation in rice of at least two transcription factors, SHORT ROOT (SHR) and SCARECROW (SCR), involved in ground tissue formation in *Arabidopsis*.

Introduction

The root anatomy of *Arabidopsis thaliana* is simple; the roots contain several concentric layers of cells, each of which assumes a specific role in the root's function (Dolan et al., 1993; Scheres et al., 1994). The function of the root epidermis, for example, involves nutrient uptake and the differentiation of root hairs, which are specialized cell structures that increase water uptake (Waisel et al., 2002). The root cap protects the growing root tip and facilitates soil penetration by secreting specific compounds. In a mature *A. thaliana* root, the radial anatomy of the root (from the outside in) consists of the epidermis, cortex, middle cortex (MC), endodermis, pericycle and vascular tissues (Fig. 1a). The cortex, MC and endodermis form the ground tissue (GT) system. The GT system and the two other tissues systems, the dermal (epidermis) and the vascular system (including the pericycle), converge to four cells in the root apical meristem (RAM) that are nearly mitotically inactive and are called the quiescent center (QC) (Evert and Esau, 2006). Each tissue system is produced following stereotypical cell divisions by an initial cell (Evert and Esau, 2006). The initial cells regenerate by first asymmetrical division and produce daughter cells that will also divide and differentiate to form root tissues. Four types of initial cells exist in the *A. thaliana* root; one population of initial cells produces the columella cells, one produces the vascular tissue, a common initial cell produces the lateral root cap and epidermis, and a common initial cell produces all of the tissues that form the GT. These initial cells surround the QC and divide less frequently than their immediate daughter cells (Campilho et al., 2006; Dolan et al., 1993). The QC forms an organizing center that is required for stem cell maintenance (Aida et al., 2004; van den Berg et al., 1997). In the 1990s, several experiments demonstrated that position but not lineage was crucial for cell fate and cell patterning (van den Berg et al., 1995, 1997). Laser ablation of all of the initial cells in contact with a QC cell induces premature differentiation of these cells (van den Berg et al., 1995). Therefore, the role of the differentiated tissue is to maintain the QC in an undifferentiated stage.

As in almost all vascular plants, *A. thaliana* contains a single endodermis layer that is characterized by the presence of Casparian bands in the radial and transverse walls of the cells (Enstone et al., 2002). The appearance of an endodermis was a key event in the evolution of land plants, and its essential function is to prevent the apoplastic passage of ions from the cortex to the stele. It is essential for the selective uptake of ions, nutrients and water, and it also has other specialized functions, such as graviperception in shoots (Tasaka et al., 1999). The function of the endodermis in apoplastic ion movement is evidenced by the multilayered endodermis that is found in several

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doi:10.1016/j.ydbio.2012.02.007

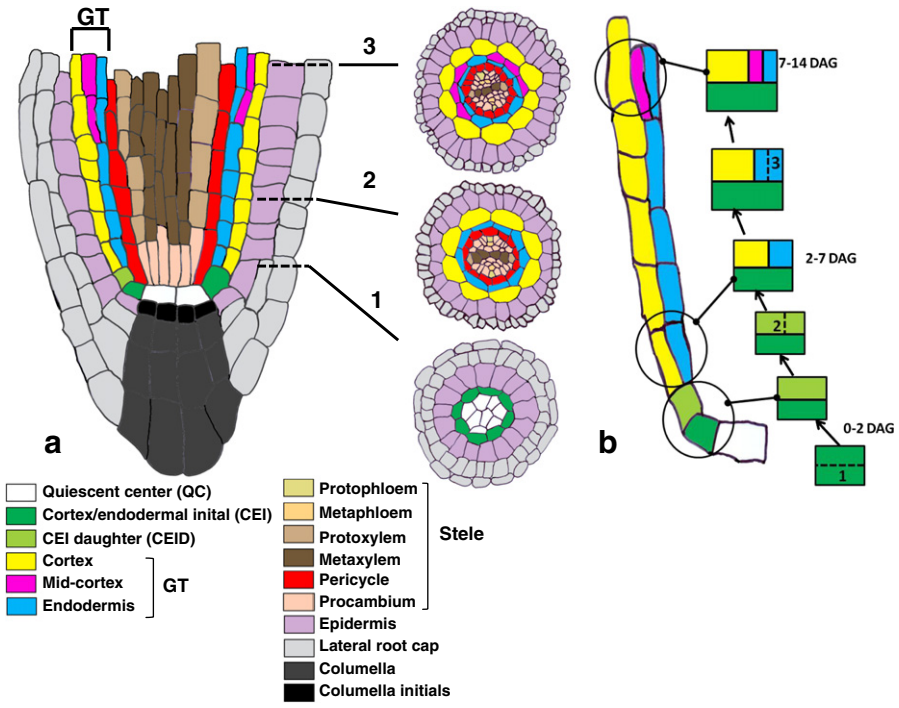

Fig. 1. *A. thaliana* root meristem structure and GT cell fate. (A) *A. thaliana* RAM structure and cell fate. Left: Longitudinal sections of the *A. thaliana* RAM. All of the cell files converge into four mitotically inactive cells, which are called the quiescent center (white). Four distinct zones of initials are present and produce all of the root tissues: vascular, including the phloem, protoxylem, metaxylem and pericycle; the lateral root cap/epidermis, with a common initial; the columella initials and the GT-containing endodermis, cortex and middle-cortex. Right: Serial root radial sections of an *A. thaliana* root at increasing distances from the QC. (1) A radial section at the QC level with the CEI visible around the QC. (2) A radial section just up to the procambium zone, which shows separate cortex and endodermis initials. (3) A radial section further from the QC in the basal meristem, where the last GT has started to appear. The middle cortex is visible between the cortex and endodermis. (B) Schematic representation of GT cell fate. The first anticlinal division (n°1) regenerates the CEI (dark green) and gives rise to the CEID (light green) during or soon after germination. The asymmetrical division of the CEID (n°2) separates the cortex (yellow) and endodermis (blue) between 2 and 7 days after germination (DAG). The late asymmetrical periclinal division of the endodermis (n°3) and the formation of the last GT layer, the middle cortex (pink), occur between 7 and 14 DAG. All of the tissues in the figure are color-coded. Adapted from Baum et al. (2002), Dolan et al. (1993), Paquette and Benfey (2005), and Petricka et al. (2009).

halophyte species. Presence of a multilayered endodermis represents an anatomical adaptation to high salt stress (Inan et al., 2004). The number of cortex cell layers is considerably larger in numerous species. These layers differentiate into more specialized tissues that reflect specific plant adaptations (Evert and Esau, 2006). In rice, a multilayered cortex differentiates into a gas-containing tissue, called the aerenchyma, which is visible in roots and represents an anatomical adaptation to water submergence (Coudert et al., 2010; Rebouillat et al., 2009). Rice can also develop a specialized GT layer, called the exodermis, that is a water barrier with a function similar to that of the endodermis (Enstone et al., 2002); this feature is shared by most vascular plants but is absent in *A. thaliana* (Enstone et al., 2002).

A fundamental problem in plant development is understanding how different cell types and cell fates are specified. GT is an elegant model that can be used to study these fundamental questions in plant roots. Identifying the mutations that affect cell fate and cell patterning is a powerful method for isolating the genes that are involved in this process. They can be identified by taking advantage of the simple and hierarchical fates of the cells that form the GT in the RAM. Numerous mutants have been isolated over the last 10 to 20 years, and characterizing their functional and molecular properties has greatly increased our understanding of the fundamental processes of plant root development. This review analyzes GT mutants to provide a detailed account of the molecular network that regulates GT formation in *A. thaliana*. The most recent results indicate an unexpectedly complex network of genes that play crucial roles in root tissue cell identity, division and differentiation and in RAM maintenance.

Ground tissue formation involves a complex interplay of transcription factors

The initial divisions that form the GT can be determined simply by looking at root anatomy (Fig. 1b). GT cell patterning has also been confirmed using ^{3}H-thymidine labeling of actively growing roots and time-lapse analyses of transgenic reporter lines (Campilho et al., 2006; Dolan et al., 1993). Cortex endodermis initials (CEIs) are formed early after seed germination. The CEIs first divide anticlinally to regenerate themselves and produce proximal daughter cells, which are called cortex endodermis initial daughter cells (CEIDs). The CEIDs divide periclinally and asymmetrically to form separate cortex and endodermis cell layers (Dolan et al., 1993). During the later root growth, at 7 to 14 days after germination (DAG), additional asynchronous asymmetric periclinal divisions occur in the endodermis to generate the MC, which is a cortical tissue (Baum et al., 2002; Paquette and Benfey, 2005). The MC appears near the protoxylem pole and progresses as a one-gyre helix (Baum et al., 2002). The identification and characterization of several GT mutants has helped to elucidate a complex genetic GT formation network that involves several transcription factors and hormonal and epigenetic control.

Transcription factors involved in cortex and endodermal identity and formation

Mutations in the *SHR* and *SCR* genes (two members of the plant-specific GRAS transcription factor family) were first identified as causing shorter roots compared to in wild type (WT) plants (Benfey

et al., 1993; Di Laurenzio et al., 1996; Pysh et al., 1999). Moreover, upon germination these mutants contain only one ground tissue layer (Di Laurenzio et al., 1996; Scheres et al., 1995) (Fig. 2). The *shr-1* monolayer becomes a cortex tissue, while the *scr-1* cell layer has a mixed endodermal and cortical cell fate (Benfey et al., 1993; Di Laurenzio et al., 1996; Helariutta et al., 2000). Interestingly, the *shr scr* double mutant phenotype is identical to the *shr* phenotype, which suggests that *SHR* is genetically positioned upstream of *SCR*. *SHR* overexpression under the SCR or the strong 35S promoter induce very large roots with supernumerary endodermal cell layers (Cui et al., 2007; Helariutta et al., 2000; Nakajima et al., 2001; Sena et al., 2004). SHR can then induce formation of supernumerary endodermis cell layers. Knocking down the *SCR* transcript using RNAi lines (scr_{rnai}) induces supernumerary CEIDs and also GT cell layers of endodermal identity (Cui et al., 2007). These results demonstrate that in GT formation, SCR is involved in CEID asymmetrical division, while SHR is involved in both CEID asymmetrical division and endodermal identity (Di Laurenzio et al., 1996; Helariutta et al., 2000; Nakajima et al., 2001; Sena et al., 2004).

Another mutant, *jkd* (*jackdaw*), was isolated in a genetic screen for *SCR*-related gene expression profiles (Welch et al., 2007). JKD is a member of a C_2H_2 zinc finger protein family (Welch et al., 2007). Ectopic random periclinal and anticlinal divisions are visible in the cortex of *jkd* mutants (Fig. 2). The inner cell from the ectopic asymmetrical division adopts endodermal characteristics. Ectopic periclinal cell divisions are not observed in double mutants (*jkd-4 scr-4* and *jkd-4 shr-2*), which suggests that *JKD* requires SHR and SCR and that SHR, SCR and JKD act in the same pathway to mediate GT formation. Ectopic anticlinal cell divisions are lacking in *jkd-4 shr-2* double mutant while still visible in *jkd-4 scr-4* suggesting both *SHR* and *SCR* are required for the ectopic periclinal cell divisions. On the other hand, only *SHR* is required for the ectopic anticlinal cell divisions in *jkd* background. *MAGPIE* (*MGP*) and *NUTCRACKER* (*NUC*) are paralogs of JKD (Levesque et al., 2006; Welch et al., 2007). However, *mgp* and the *mgp nuc* double mutant do not have any obviously altered phenotype, which suggests that a high level of redundancy exists among family members (Fig. 2). Moreover, the *MGP* RNAi (mgp_i) lines in the *jkd-4* background suppress the ectopic cell division that is present in *jkd-4*, which indicates that MGP antagonizes JKD.

SCHIZORIZA (SCZ) encodes an HSFB4 (heat shock transcription factor B4), which suggests that some heat shock transcription factors play an unexpected key role in cell fate and cell identity. In *scz* mutants, an additional subepidermal GT layer with a mixed epidermal/cortical/lateral root cap identity is formed (Fig. 2) (Mylona et al., 2002; Pernas et al., 2010; ten Hove et al., 2010). This supernumerary layer results from the abnormal activity of the CEI, which first divides periclinally during embryogenesis (Mylona et al., 2002; Pernas et al., 2010; ten Hove et al., 2010). The CEIs also divide anticlinally and then periclinally to form two or more GT layers with mixed cell fates for *scz-1* and for the stronger *scz-2* alleles respectively (Pernas et al., 2010; ten Hove et al., 2010). The outer GT cell layers of these mutants express epidermal and cortical markers, and the inner GT cell layer has an endodermis-like identity (ten Hove et al., 2010). However, *SCZ* overexpression induces supernumerary CEIDs by promoting anticlinal cell divisions (Fig. 2) (Pernas et al., 2010; ten Hove et al., 2010). Therefore, SCZ promotes anticlinal CEI divisions and inhibits asymmetrical CEID divisions. Moreover, this gene has a critical role in cell fate separation in ground and other tissues. Surprisingly, the *scr-4 scz-1* double mutant lacks GT (Fig. 2) (Pernas et al., 2010). *SCZ* and *SCR* interact genetically, and this interaction is crucial for GT formation (Mylona et al., 2002; Pernas et al., 2010).

Transcription factors involved in middle-cortex formation

During later root development, additional endodermal periclinal divisions generate the MC. MC formation may be altered in some mutants. Indeed, the *scr* and *shr* mutants were again observed to display different phenotypes; the periclinal division of the endodermis that produces the MC is absent in *shr* mutants and appears more precociously in *scr* mutants (Paquette and Benfey, 2005; Wysocka-Diller et al., 2000). Therefore, SCR is not required for MC formation, but it does delay the timing its formation (Fig. 3). Mutations that affect hormonal factors, including the root growth factor gibberellin (GA), its antagonist abscisic acid (ABA) (Fu and Harberd, 2003; Hoffmann-

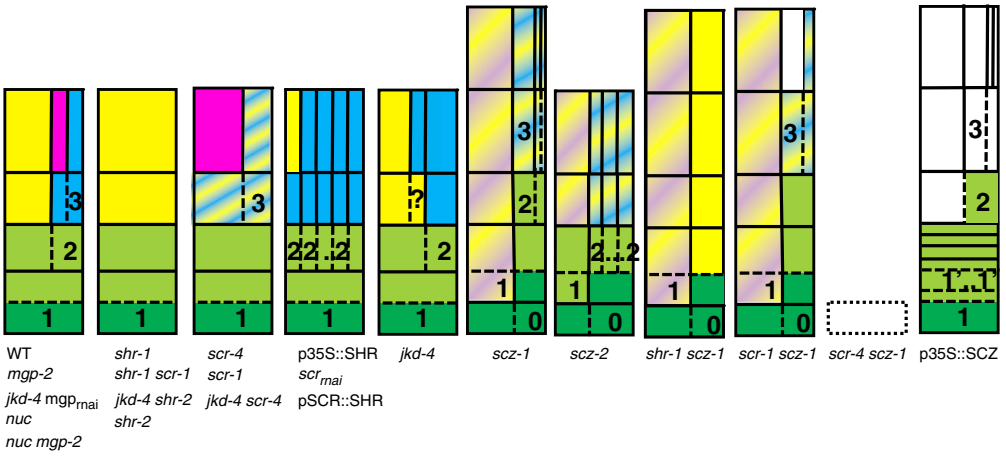

Fig. 2. Schematic representation of GT cell fate in several GT mutants. In *shr*, no asymmetrical formative division (n°2 and n°3) is observed, and consequently, one cell layer is visible instead of three, as in the WT. The single layer has a cortex identity, which indicates that *SHR* is also needed for endodermal identity. Due to the absence of the second asymmetrical division, no MC is formed in *shr*. In *scr*, the first asymmetrical division is lost (n°2), and the monolayer has a mixed cortical–endodermal cell identity, which suggests that *SCR* plays a role in the first asymmetrical division but not in cell specification. Moreover, the second asymmetrical division (n°3) is visible in *scr* and appears precociously compared to the WT, suggesting that *SCR* negatively affects MC formation. The *scr shr* double mutant has a phenotype similar to that of *shr*, confirming that *SHR* acts upstream of *SCR*. The *scz* mutant has an additional asymmetrical periclinal division (n°0) that is not found in the WT and that generates the CEI and has a mixed cell fate layer with a mixed epidermis and cortex identity. No CEIs are formed in the *scz scr* double mutant, and no GT tissues are visible between the epidermal and vascular tissues. P35::SHR, pSCR::SHR and RNAi SCR all contain supernumerary asymmetrical cell divisions that generate a cell layer of endodermal identity. The *jkd* mutant has abnormal asymmetrical divisions of the cortex, which generate an ectopic, random cell of endodermal identity. *mgp* mutants do not display any phenotype, but the down-regulation of *MGP* (mgp_i) in the *jkd* background restore the WT GT cell fate. The tissues are color-coded as in Fig. 1. The hatched lines indicate mixed-cell identity, and the hash colors refer to the different identities of the tissues that are visible in the cells (purple indicates an epidermal identity). The numbers refer to the division sequence of the GT initials and follow the nomenclature of Fig. 1b.

Benning and Kende, 1992) and ethylene, also affect MC formation (Cui and Benfey, 2009a). Paquette and Benfey (2005) were the first to show that treating WT roots with GA delays MC. However, inhibiting GA biosynthesis by treating WT roots with paclobutrazol (PAC) or through *ga1-3* mutations reveals that the MC of WT roots develops earlier in the absence of GA (Paquette and Benfey, 2005). Therefore, both the GA and SCR pathways inhibit MC formation. Moreover, the weak *ga1-3* phenotype is largely enhanced in *ga1-3 scr-5* double mutants, and chemical treatment also enhances the observed phenotypes in the *scr* mutant background. These results suggest that GA and SCR use partially independent pathways to fulfill their functions (Heo et al., 2011). Additional GA signaling mutants have also been studied (Cui and Benfey, 2009b). For example, GA receptor (*gid1*) and GA-insensitive (*rgaΔ17*) mutants, both of which precociously form the MC, confirm the role of GA in MC formation (Cui and Benfey, 2009b). Interestingly, WT roots that are treated with ABA never form an MC (Cui and Benfey, 2009b).

The MC is also under epigenetic control; inhibition of histone deacetylation (HDAC) gained mutation in *lhp1-4* (like heterochromatin protein 1) induces premature MC formation (Cui and Benfey, 2009b) (Fig. 3). SPY is an O-linked N-acetylglucosamine (GlcNAc) transferase (OGT) that influences cytokinin and GA signaling in plant (Greenboim-Wainberg et al., 2005; Silverstone et al., 2007). In animal, OGTs interact with HDACs (Yang et al., 2002) but it remains to be demonstrated in plants. In *spy* (spindly), MC formation is also earlier (Cui and Benfey, 2009b) but the mechanism affecting MC formation in spy, either hormonal or epigenetic, is unclear. To confirm the role of HDAC in MC formation, WT roots have been treated with Trichostatin A (TSA), which is a specific HDAC inhibitor; this treatment also caused premature MC formation. The phenotype is not caused by GA reduction because simultaneous TSA and GA treatments did not alter the TSA effect on MC formation. Together, these observations suggest that the SCR, LHP1, SPY and GA pathways act together to modulate the timing of MC formation through a convergent network in which GA plays a key role.

Recently, SCL3, another member of the GRAS plant-specific transcription factor family, has also been found to play a role in MC formation (Heo et al., 2011). In *scl3*, the MC appears precociously; conversely, the MC appears late in *SCL3*-overexpressing lines. Several mutant combinations, such as *scl3 ga1-3*, *scl3 scr* and *scl3 scr rga* (see Fig. 3 for details), induce precocious MC formation, and the triple mutants display the most severe phenotypes. These results suggest that SCL3, SCR and GA act together to regulate MC formation and that SCL3 is one of the key places where GA, SCR and SHR converge.

An integrated model for the root ground tissue network

The mechanisms of action of SHR and SCR, the two main players whose proteins are transcribed from embryogenesis to the time of MC formation, have now been extensively studied and described (Iyer-Pascuzzi and Benfey, 2009). A schematic model that includes most of the results that have accumulated over the last approximately 15 years is shown in Fig. 4.

In GT formation

SHR and SCR are both required to the asymmetrical division of the CEID and proper GT formation; however, SCR is also involved in inhibiting the subsequent divisions (Paquette and Benfey, 2005) to maintain a two-layered GT (Fig. 4). Complemented lines of *shr* and *scr* mutants containing inducible promoters have demonstrate that SHR directly activates transcription of *CYCD6;1*, a cyclin D-type (Sozzani et al., 2010). *CYCD6;1* is expressed in the CEI and appears to act redundantly with cell cycle regulators such as the cyclin dependent

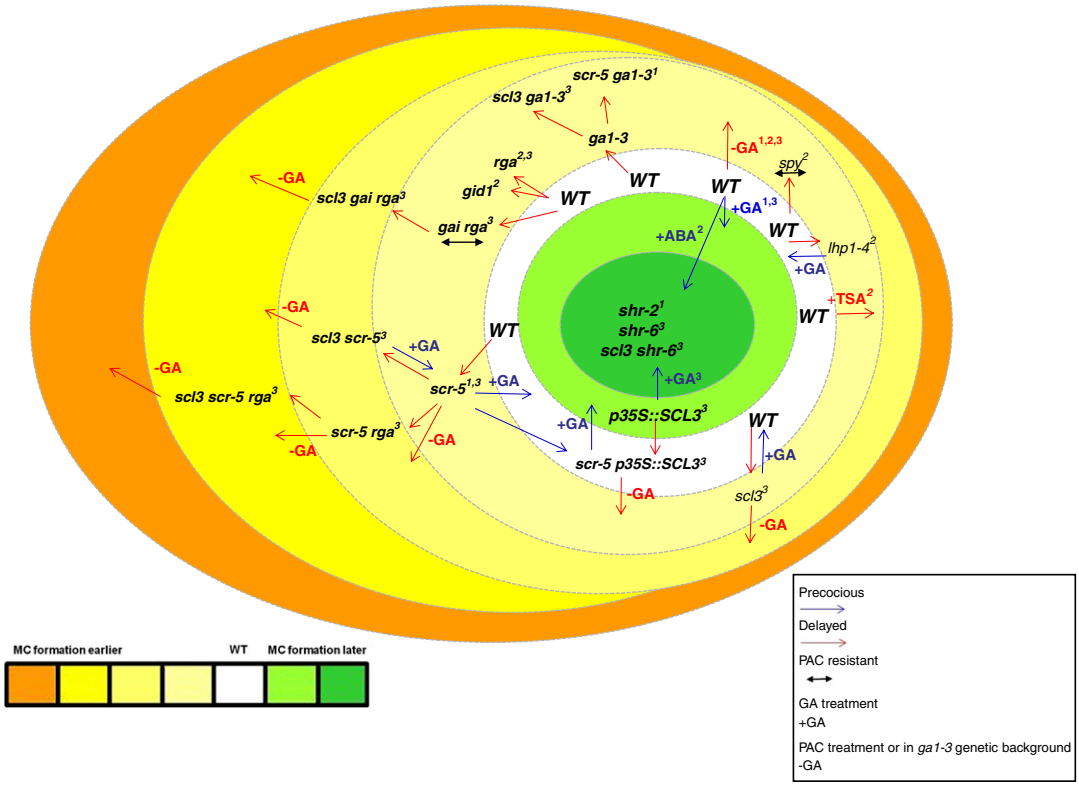

Fig. 3. Timing of MC formation. In the WT, the MC appears between 7 and 14 days after germination (DAG) (white). An interplay between transcription factors, hormones and epigenetic regulations control MC formation. *scr*, *scl3*, *lhp1*, *spy* mutants, the GA signaling mutant and PAC (−GA) and TSA treatment induce precocious MC formation (light yellow). The experiments that combined these pathways (double mutant, triple mutant and mutant treatments) resulted in exceptionally precocious MC formation (yellow to orange). Conversely, GA treatment and *SCL3* overexpression delayed MC formation (light green). Finally, *shr* mutations and ABA treatment result in no MC (dark green). 1—Paquette and Benfey (2005); 2—Cui and Benfey (2009b); 3—Heo et al. (2011).

kinases *CDKB2;1* and *CDKB2;2*, to induce asymmetrical cell divisions in the CEI (Sozzani et al., 2010). As was mentioned earlier, ectopic SHR overexpression and the SCR_{rnai} mutant lines result in supernumerary CEID and GT cell layers of endodermal identity. This phenotype can be explained because the function of SCR is to sequester SHR in the nuclei of these cells and prevent its movement to the outer layers. This hypothesis is supported by several observations. The SHR gene is primarily expressed in stele. From there, the protein moves one cell layer outward on the radial root axis to the QC, CEI, CEID and endodermis, a movement that is necessary for proper root patterning (Carlsbecker et al., 2010; Cui et al., 2007; Gallagher and Benfey, 2009; Helariutta et al., 2000; Nakajima et al., 2001). SHR movement requires cytoplasmic and nuclear subcellular localization in the stele and nuclear in endodermis, which suggests a mechanistic link between its nuclear transport and cell-to-cell movement (Gallagher and Benfey, 2009). The leucin heptad repeat II domain of the SHR protein (LNELDV) has been shown to be critical for its movement but not for its function (Gallagher and Benfey, 2009). SHR acts upstream of SCR by promoting direct transcription of the SCR gene (Cui et al., 2007; Helariutta et al., 2000; Levesque et al., 2006) even if SCR is not entirely dependent upon SHR for expression (Heidstra et al., 2004; Helariutta et al., 2000; ten Hove et al., 2010). Indeed, SCR is mainly expressed in the QC, CEI, CEID and endodermis (Di Laurenzio et al., 1996). There is also expression in the cortex, but at a lower level than in the endodermis. Moreover, it has been shown that the SHR and SCR proteins interact and that this complex is part of a positive SCR transcription loop (Cui et al., 2007; Levesque et al., 2006). Thus, the high level of SCR protein that is produced by both regulation modes permits formation of a nuclear SHR/SCR complex, which sequesters and limits movement of the SHR protein.

In the *scr* background, SHR is cytoplasmic in the single GT layer and can move beyond this layer (Nakajima et al., 2001; Sena et al., 2004). Moreover, SHR movement and the supernumerary cell layers are inversely proportional to the level of SCR expression in scr_{rnai} mutant lines. Therefore, the current model for GT formation relies on maintaining the proper equilibrium between SHR and SCR levels. If SHR is not completely sequestrated by SCR, the SHR protein moves behind the single endodermal cell layer and activates *SCR* expression in that layer, which allows another asymmetrical division, another movement to the extra layer, and so on (Cui et al., 2007; Helariutta

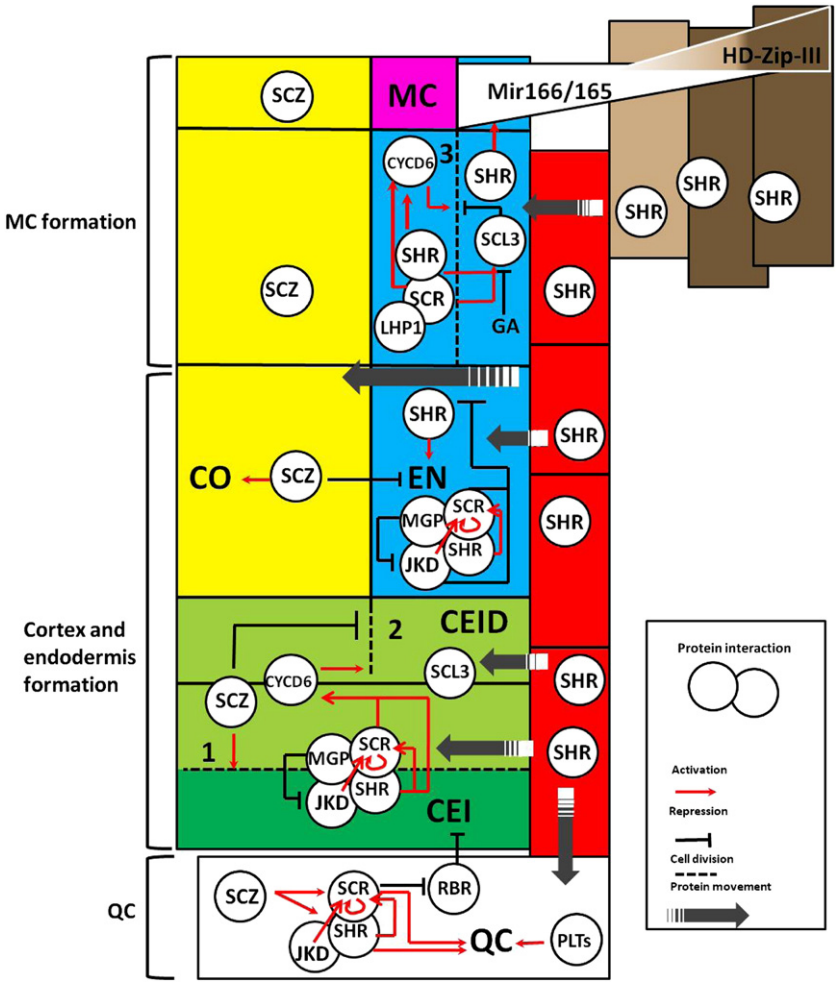

Fig. 4. A molecular model for the GT genetic network. A schematic view of the GT protein network interactions and transcriptional regulations. The *SHR* transcripts are produced in the stele (pericycle in red) before the proteins move to the CEI (dark green) and CEID (light green). In the CEID, SHR activates *SCR*, which maintains a high expression level through autoactivation of its own transcription, and *SHR* and *SCR* both induce the formative division that separates the CEI and CEID initial by direct transcriptional activation of *CYCD6;1* (n°2) and indirect activation of other cyclins. SCR interacts with SHR, and both proteins are targeted to the nucleus, where SCR prevents SHR from moving behind the endodermis. JKD also limits SHR movement by increasing the *SCR* level. MGP interacts with SHR, SCR and JKD; MGP also antagonizes JKD by inhibiting it and transcriptionally activated by SHR and SCR. During MC formation, SHR activate transcription of CYCD6;1 and other cyclins to induce the last formative periclinal division (n°3), while SCR inhibits iMC formation. SCL3 inhibits MC formation and is itself negatively regulated by GA and positively regulated by SCR and SHR. Movement of the SHR protein in the endodermis induces miRNA165/166 transcription, which generates a gradient that extends into the stele. Several transcription factors from the HD-ZIPIII class are targeted for degradation by miRNA165/166, which generates a transcriptional gradient inside the stele. A high dose specifies metaxylem, and a low dose specifies protoxylem. SCR and SHR are both needed to maintain a functional QC. PLT is also independently required for QC formation and specification. SCR inhibits RBR expression indirectly while RBR repress stem cell formation. SCZ promote transcription of SCR and SHR in QC together with JKD.

et al., 2000; Nakajima et al., 2001). In WT plants, the SHR/SCR complex is rapidly inactivated following the asymmetrical CEID division. In the cortex, SHR and SCR are presumed to be quickly degraded, while the SHR-inductive capacity of SCR is lost in the endodermis (Heidstra et al., 2004).

Recently, a novel SHR interacting protein, SIEL (SHORT-ROOT INTERACTING EMBRYONIC LETHAL), was identified (Koizumi et al., 2011). SIEL interacts also with three transcription factors, CAPRICE, TARGET OF MONOPTEROUS 7 and AGAMOUS LIKE 21 all known to move from cell to cell. SIEL localizes to cytoplasm and nucleus in roots and is associated with endosomes. SHR and SCR regulate SIEL expression forming an additional feedback loop regulating SHR movement (Koizumi et al., 2011).

Several results have suggested that the *SCR*, *LHP1* and GA pathways act together, through a convergent network in which GA plays a key role, to modulate the timing of MC formation (Cui and Benfey, 2009b). First, the precocious MC formation phenotype can be observed in *lhp1-4* and *scr*. Second, SCR and LHP1 interact in a yeast 2-hybrid assay. Precocious MC formation in *scr* (Paquette and Benfey, 2005) and *lhp1-4* (Cui and Benfey, 2009b) mutants can be suppressed by GA; in the *scr* mutants it is enhanced by PAC, which confirms the antagonistic action of *SCR*/*LHP1* and GA in MC formation. SCL3, another member of the GRAS transcription factor family, is a direct target of both SHR and SCR (Cui et al., 2007; Levesque et al., 2006) and has the same expression pattern as *SCR* (Heo et al., 2011; Pysh et al., 1999; Zhang et al., 2011). It has been shown that *SCL3* is down-regulated by GA and up-regulated by the SHR/SCR complex (Heo et al., 2011; Zhang et al., 2011). Precocious MC formation in *scl3* mutants is enhanced by GA-deficient conditions. SCL3 seems to be one of the key nodes at which GA and the SCR and SHR pathways converge; in addition, it is responsible for other functions in GA's tissue-specific control of growth and division (Heo et al., 2011; Zhang et al., 2011).

Recent data have shown that in addition to SHR and SCR, MGP and JKD are involved in GT specification. All of these proteins interact with each other; yeast 2-hybrid experiments have demonstrated the interaction of SCR with SHR, of SHR with JKD, and of MGP with SCR, SHR and JKD (Cui et al., 2007; Welch et al., 2007). This GT genetic network restricts SHR movement and is controlled by transcriptional feedback from these interacting partners (Cui et al., 2007; Helariutta et al., 2000; Levesque et al., 2006; Welch et al., 2007). For example, JKD promotes SCR expression and helps to inhibit SHR movement because SHR can migrate one layer outward in the *jkd* background (Welch et al., 2007). MGP has also been shown to be a direct target of SHR and SCR, and SCR and MGP are direct targets of SHR (Cui et al., 2007; Levesque et al., 2006). MGP, which plays an antagonistic role to JKD, seems to act primarily by inhibiting JKD transcription (Welch et al., 2007). Therefore, JKD and MGP are involved in setting the boundaries between the endodermis and cortex. JDK binds to *SCR* and *MGP* promoter in protoplasts and yeast one-hybrid assays. Moreover, promoter activities are enhanced by addition of SHR and SCR with JKD suggesting JKD regulates *SCR* and *MGP* in cooperation with SHR, SCR and MGP (Ogasawara et al., 2011).

The *SCZ* heat shock factor is expressed in GT stem cells, primarily in the cortical layer. Its role is to promote CEID formation, to suppress premature asymmetric division of the CEID into cortex and endodermis and to promote cortex identity. Indeed, *SCZ* overexpression promotes ectopic cortex identity in other root tissues (Pernas et al., 2010; ten Hove et al., 2010). *SCZ* also represses epidermal and lateral root cap identity in the GT. Therefore, SCZ promotes cell fate separation non cell-autonomously from cortex cells. Both the protein and mRNA are expressed only in the cortex, which suggests that SCZ acts by an unknown intermediary factor to promote cell fate separation in the cell layers surrounding the cortex [20]. SCR and SCZ are both required for CEI formation, but neither the nature of their interaction nor the molecular mechanism that is used by *SCZ* to promote cell fate separation in numerous initial cells from its primary transcription site in the cortex is known.

In the QC

In addition to their functions in root patterning and endodermal identity, the *SHR* and *SCR* genes are also necessary for QC identity and maintenance (Fig. 4) (Di Laurenzio et al., 1996; Helariutta et al., 2000; Sabatini et al., 2003; Wysocka-Diller et al., 2000). To fulfill these functions, the SHR/SCR complex modulates the expression of and interacts with various partners. This complex formed by all interacting partners differs among several different cell types adding another level of complexity (Welch et al., 2007). The *scr* and *shr* mutants develop short roots due to determinate root growth. Several QC markers are not expressed in *scr-1* mutants, and markers for differentiated columella are ectopically expressed in columella initials, which suggests a partial loss of QC identity. Moreover, SCR is required for its own transcription in the QC because GFP is not visible in the QC when a pSCR::GFP transcriptional fusion is transformed into *scr-1* mutant plants (Sabatini et al., 2003). Complementary root growth and meristem maintenance is achieved only when SCR is expressed in the QC, which demonstrates that SCR is required cell autonomously to promote QC identity and maintenance. The *shr-1* and *scr-1* mutants display a similar root growth and meristem maintenance defects albeit *shr-1* roots are considerably shorter and terminate the meristem earlier than *scr* ones. Complementation of *shr-1* determined root growth phenotype cannot be achieved by SCR expression in the *shr-1* QC. This result confirms that SCR and SHR are both needed for QC identity and RAM initial cell maintenance (Sabatini et al., 2003). Expression of cell autonomous SHR or SCR in stele can rescue root length of *shr* and *scr* mutants suggesting the signals do not necessary have to come from QC (Gallagher and Benfey, 2009).

SCR plays a non-cell-autonomous role in maintaining RAM initial cells by regulating the retinoblasta related (RBR) pathway. RBR is a key point of entry into the G1 phase of the cell cycle. There is an increase in the number of stem cells in RBR-deficient mutants, while the stem cells differentiate quickly and consume the root meristem in overexpressing lines (Wildwater et al., 2005). Therefore, RBR controls the level of stem cell *versus* differentiated cell populations to maintain a functional RAM. An inducible root-specific reduction of RBR in the *scr-4* genetic background restores partially functional initial cells. It has been suggested that *SCR* may limit the RBR pathway by downregulating Kip-related proteins or upregulating D cyclins and that it may therefore act in the G1–S regulatory pathway (Wildwater et al., 2005).

Two other genes, *PLETHORA1* (*PLT1*) and *PLETHORA2* (*PLT2*), act in parallel with *SCR* and *SHR*. They encode AP2-type putative transcription factors and are redundantly required for maintaining root stem cells (Aida et al., 2004). PLT proteins have been showed to affect stem cell identity in a dosage dependent manner, with higher levels specifying the root stem cell niche (Aida et al., 2004). *PLT* overexpression induces ectopic formation of root QC in the shoot in cell layers adjacent to pericycle. QC appears then exactly at the site where SHR and SCR are present (Aida et al., 2004), which confirms that the *SCR*/*SHR* and *PLT* genes are both required for QC specification and primordia formation.

SCZ is expressed in the QC (Pernas et al., 2010), and the *scz-2* allele has been independently isolated when screening for mutants that affect QC specification and stem cell formation (ten Hove et al., 2010). Its role in the QC seems to be facilitating cell fate separation between the QC and columella stem cells because both display a mixed cell fate. However, *SCZ* is not critical for the stem cell niche because even in the strong *scz-2* allele, the roots still display indeterminate growth (ten Hove et al., 2010). Finally, SCZ is required for *SCR* and *SHR* expression in the QC, and it promotes their transcription (ten Hove et al., 2010).

For protoxylem versus metaxylem formation in vascular tissues

In *A. thaliana*, the vascular tissues are surrounded by the pericycle and form the stele, which is externally limited by the endodermis. In *scr* and *shr* mutants, metaxylem ectopically differentiates in place of protoxylem, which indicates that *SHR* and *SCR* affect xylem development (Carlsbecker et al., 2010; Levesque et al., 2006). SHR can move from the stele to the endodermis, where it activates transcription of miRNAs (miR165/166). mir165/166 moves from the endodermis to the stele, which generates an miRNA gradient. Several transcription factors of the HD-ZIP-III class are targeted by miR165/166, and an HD-ZIPIII expression gradient in the root meristem is inversely correlated with miR165/166; it is highest in the center of the xylem axis and lower towards the stele periphery (Carlsbecker et al., 2010; Miyashima et al., 2011). This level determines the xylem type and the formation of the xylem itself. A high dose specifies metaxylem, and a low dose specifies protoxylem (Carlsbecker et al., 2010; Miyashima et al., 2011). miR165/166 normally acts to exclude HD-ZIPIII mRNA from the stele periphery and from the GT. The ectopic production of protoxylem instead of metaxylem is found in a HD-Zip III triple mutant, and no xylem differentiation can be detected when the 5 HD-ZIP III members are disrupted (Carlsbecker et al., 2010). The miRNA-dependent suppression of HD-ZIPIII genes is required for protoxylem specification and correct pericycle differentiation (Miyashima et al., 2011).

Future trends: moving back and forth between plant models

GT network evolution studies have provided a unique perspective for following the emergence and diversification of developmental pathways along the evolutionary path of land plants. In all angiosperms, GT formation results from the successive divisions of a common initial cell. A major difference exists nevertheless between GT formation in monocots and GT formation in dicots. The GT and epidermis belong to a single common initial in monocots, while in dicots, the lateral root cap and epidermis share a common initial cell (Clowes, 1994). In monocots, the first asymmetrical division of the CEID produces the epidermis, and the subsequent asymmetrical cell divisions generate the cortex and endodermis. The CEID should then be renamed Common Epidermis Endodermis Initial Daughter cell (CEEID) (Fig. 5). GT formation in rice, the model monocot plant, represents an intriguing contrast to *A. thaliana.* In rice, the roots system is made of embryonic and post-embryonic roots. During germination, a radicle emerges followed a few days later by many adventitious post-embryonic crown roots (Coudert et al., 2010; Rebouillat et al., 2009). All roots can bear lateral roots made of two types, the small and large lateral roots. Radial anatomy of all rice root types follow a similar organization with, from outside in, one layer of epidermis, one of exodermis, one of sclerenchyma, from zero to more than 12 cortical cell layers, one layer of endodermis and the stele tissues. All root types differ usually in diameter and this is strictly correlated with the number of cortical cell layers. For instance, large lateral roots have 3 cortex cell layers while small lateral roots do not have any. Variation in the number of cortex cell layers is observed between the five root types and during rice development. These variations result from a variable number of periclinal cell divisions of the CEEID (Fig. 5).

There should be a control mechanism for the number of cortical cell layers and the subsequent periclinal cell divisions of the CEEID in rice, and it remains to be determined whether any of the identified *A. thaliana* GT network members are involved in this process. Alternatively, it is possible that a completely different molecular mechanism exists in rice. The first available results, which were based on *in situ* hybridization (Cui et al., 2007; Kamiya et al., 2003; Lim et al., 2000) and yeast 2-hybrid experiments with the OsSHR1 and OsSCR1 orthologs (Cui et al., 2007) have suggested conservation of function of *OsSHR1* and *OsSCR1* in rice in endodermis formation. *OsSHR1* expression is restricted to the stele (Cui et al., 2007) like *AtSHR* in *A. thaliana* while *OsSHR2* seems to expand in endodermis and some cortex cell

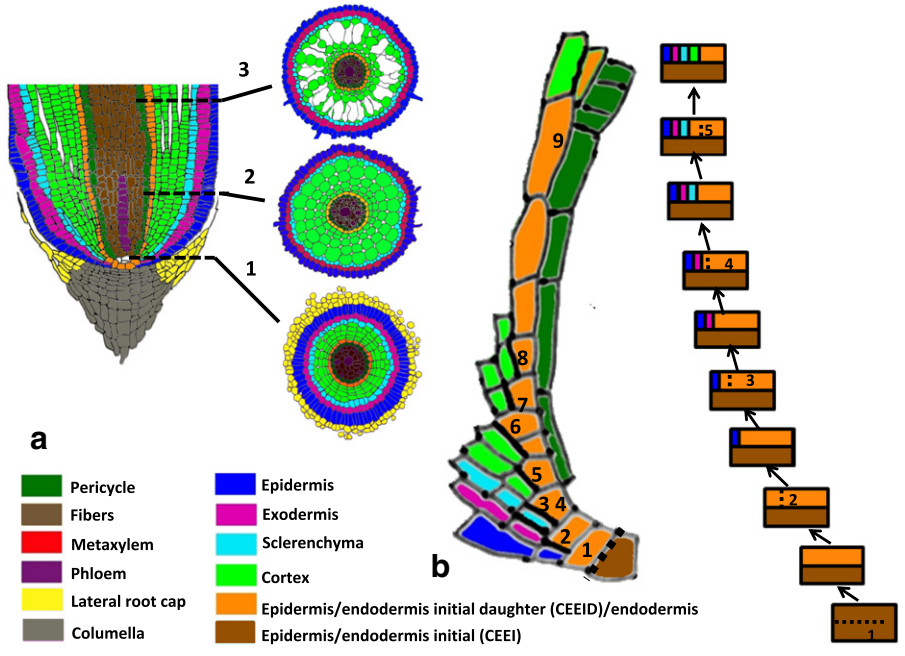

Fig. 5. Rice root meristem structure and GT cell fate. (A) Rice RAM structure and cell fate. Left: Longitudinal sections of the rice RAM. Three distinct zones of initials are present and produce all of the root tissues: vascular, including the phloem, protoxylem, metaxylem and pericycle; the columella and lateral root cap and the CEEI containing epidermis, exodermis, sclerenchyma, cortex and endodermis. Right: Serial root radial sections of a rice radicle at increasing distances from the QC. (1) A radial section at the QC level with the periclinal divisions of CEEID still visible. (2) A radial section just up this zone, with all cell layers visible. (3) A radial section further where cortex is mature and has started to differentiate aerenchyma. (B) Schematic representation of epidermis/GT cell fate for a radicle. The first anticlinal division (n°1) regenerates the CEEI (brown) and gives rise to the CEEID (orange). The asymmetrical division of the CEEID (n°2) separates the epidermis (purple) and endodermis (orange). Successive periclinal divisions generate exodermis (n°3), sclerenchyma (n°4), the first layer of cortex (n°5) and four more cortex layer (n°6, 7, 8, 9). The number of cortex layer formed is variable among rice root types. All of the tissues in the figure are color-coded. Adapted from Coudert et al. (2010) and Rebouillat et al. (2009).

layers (Kamiya et al., 2003). Yeast 2-hybrid experiments confirm interaction of OsSHR1 with AtSCR and OsSCR1 suggesting conservation of function at least for OsSHR1 and OsSCR1 in rice while OsSHR2 has probably a more divergent function in rice root development; however, no *in planta* functional analyses of their functions are currently available.

A large set of genes and hormonal conditions modulate MC formation in *A. thaliana*. The high plasticity of MC formation suggests that the multilayered GTs that exists in several angiosperms may have originated at various locations in the GT regulation network. Consistent with this hypothesis, identifying the functions of key gene, including SCR, SCL3, GAI orthologs, and hormonal effects on cortex formation in species with a multilayered cortex should help to confirm or invalidate the role of the MC in forming the multilayered cortex and GT.

Soon after cell division of the CEI, both daughter cells are locked in two different cell fates. Such mechanism includes desensitization of the SCR promoter to SHR inducibility in daughter endodermis cell. This mechanism does not involve variable level of SCR expression between CEID and daughters. Neither does it deal with a critical amount of SCR for cell division. So, desensitization is needed to insure that SHR proteins still present in endodermis daughter cell do not induce SCR expression again and result in a second formative division (Heidstra et al., 2004). If *SCR* promoter desensitization is lost or affected, it can provide a plausible explanation for the formation of multiple cortex layers in other species. When SHR is overexpressed in *A. thaliana* RAM initial cells, additional cell layers with mixed cell fates are formed because SHR also promotes endodermal identity. In *A. thaliana*, SHR has two functions in GT formation: endodermis specification and the promotion of the CEID asymmetrical divisions. Rice contains two SHR orthologs (Cui et al., 2007; Kamiya et al., 2003). If this dual function is separately assumed by the two rice SHR orthologs (subfunctionalization), modulating SHR ortholog expression in the CEEIDs can induce variations in the number of cell divisions and, ultimately, in the number of cortical cell layers. Finally, the mechanism that produces multiple cortex layers may be completely distinct.

The increased availability of molecular tools for these two model species and the refinement of the network that is involved in *A. thaliana* GT formation will help to generate results that answer fundamental plant development and evolutionary questions.

Acknowledgments

We wish to acknowledge two anonymous referees for their comments and critics that contribute to greatly improve manuscript quality.

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