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Key words: coring, fine root, isotope, longevity, minirhizotron, root turnover.

Letters

The endodermis: a horsetail's tale

Cui *et al.* (2007) provided a compelling explanation of the mechanism controlling endodermis specification in the root of *Arabidopsis*. The mechanism consists of a positive feedback loop that involves the transcription factors SHORTROOT (SHR) and SCARECROW (SCR). According to Cui *et al.*'s (2007) model, endodermal specification is achieved by export of SHR from the root stele into endodermis precursors that express *SCR*. There, SHR binds to SCR and together they promote production of more SCR, which traps all SHR exported from the stele so that none can escape beyond the endodermal layer to trigger the formation of additional endodermises. Interestingly, Cui *et al.* (2007) also identified potential functional homologs of *SHR* and *SCR* in the rice root. Based on these findings, they suggested that an SHR-SCR feedback loop-type mechanism of endodermal specification might have been acquired early in land plant evolution and could be conserved across most vascular plants. If this were true, then Cui *et al.*'s (2007) model could apply to the development of the endodermis in most, if not all, tracheophytes. The identification, in *Pinus sylvestris* roots, of an SCR putative homolog expressed in the quiescent center equivalent (stele and root cap initials) and in the endodermis (Laajanen *et al.*, 2007) suggests that SCR has comparable functions (maintenance of root radial patterning and endodermal specification) in gymnosperms and angiosperms. This indicates that Cui *et al.*'s (2007) model could indeed apply at least to all seed plants, and provides additional impetus for the hypothesis of a conserved mechanism controlling endodermal specification across all vascular plants.

Discussing this exciting possibility, Dolan (2007) pointed out that there are some rare exceptions from the simple structure

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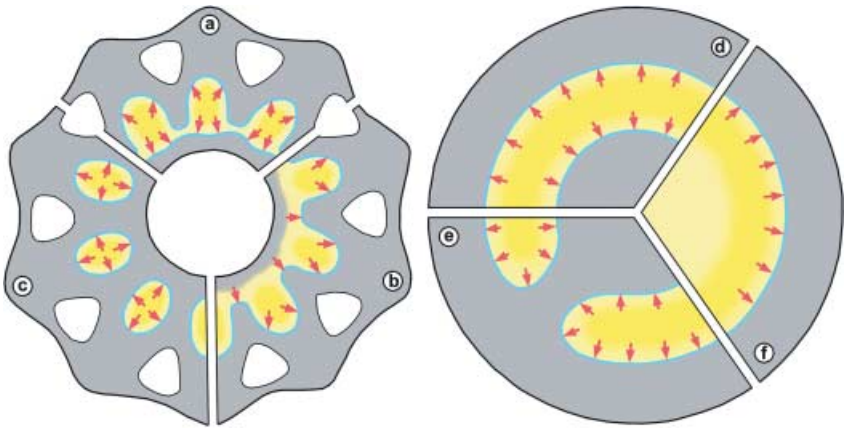

Fig. 1 Application of an SHR-SCR positive feedback loop-type mechanism of endodermis specification, hypothetically conserved across vascular plants, to explain the variety of endodermal structures of vascular plants. Yellow, vascular tissue, procambium, nonconducting tissue of vascular/procambial specification or general site of *SHR* homolog expression. Blue, endodermis or site of *SCR* homolog expression. Red arrows, direction of export of SHR homolog. (a) *Equisetum* stem internode, bi-endodermal; (b) *Equisetum* stem internode, outer endodermis only; (c) *Equisetum* stem internode, individual endodermal layers for each vascular bundle; (d) *Equisetum* stem node, bi-endodermal; (e) siphonostelic fern stem, bi-endodermal, with leaf gap; (f) monocot root and stem of fern with outer endodermis only.

with one endodermal layer seen in the *Arabidopsis* root. He cited the stems of some *Equisetum* (horsetail) species that develop two endodermal layers, suggesting that 'these horsetails have apparently tinkered with their *SCR* and *SHR* genes', and intimating that some variation in Cui *et al.*'s (2007) model based on *Arabidopsis* was needed to explain endodermal specification in *Equisetum*. A close look at the anatomy of *Equisetum* demonstrates that if the mechanism uncovered by Cui *et al.* (2007) is indeed conserved across vascular plants, then the double endodermis of *Equisetum* does not necessitate significant departures from that model, providing additional support for it. Discussion of this alternative opens new perspectives, outlined later in this paper, for understanding other types of endodermal structure encountered frequently across the spectrum of plant diversity and which break away from the anatomical norm established by the roots of dicotyledonous angiosperms.

Two endodermal layers

In Cui *et al.*'s (2007) model, endodermal specification is achieved by active export of SHR from the stele located at the center of the root, centrifugally into endodermis-cortex precursors that express *SCR*. In bi-endodermal stems of *Equisetum*, the two endodermal layers (outer and inner endodermis) develop on either side of a ring of vascular tissue, whether the latter is dissected into discrete bundles (in internodes; Fig. 1a) or not (at nodes; Fig. 1d) (Ogura, 1972). If the same source-sink pathway of SHR transport demonstrated in *Arabidopsis* functions in horsetails, then given the position of vascular tissues between the two endodermal layers, no alteration is necessary for Cui *et al.*'s (2007) model to explain this structure. In these bi-endodermal stems, the SHR homolog would simply have to be exported away from the vascular tissues, just as in Cui *et al.*'s (2007) model. And since, in these *Equisetum* stems, the vascular tissues do not occupy the center of the stem, export of SHR homolog away from the vascular tissues would be bidirectional – centrifugal and centripetal (Fig. 1a,d). If the *SCR* homolog is expressed on both sides of the ring of vascular tissue simultaneously, then together the two processes would result in specification of two endodermal layers.

The bi-endodermal structure of *Equisetum* is far from being a rare exception. Similar structures characterize the stems of most ferns with a siphonostelic architecture (as defined by Beck *et al.*, 1982; see also Bower, 1935; Ogura, 1972). If an SHR-SCR feedback loop-type mechanism is plesiomorphic and conserved across all vascular plants, then the development of an outer and inner endodermis could be explained in all these plants by the same processes as described earlier: export of SHR homologs from, and expression of *SCR* homologs around, the hollow cylinder of vascular tissue, both centrifugally and centripetally (Fig. 1e). Indeed, there is no reason why SHR would not be exported away from the vascular tissue in all directions; and in fact, this is what happens in the *Arabidopsis* root, where SHR is exported into neighboring tissue all the way around the vascular tissue.

Discrete vascular strands with individual endodermal layers

The same model (export of SHR away from vascular tissue in all directions) could also explain the anatomy of *Equisetum* stems that exhibit an individual endodermal layer around each vascular bundle (Fig. 1c). Given the organization of the vascular tissue of *Equisetum* internodes into discrete strands, this endodermal arrangement makes even more sense in light

of Cui *et al.*'s (2007) model, requiring the same type of centrifugal export of SHR homolog around each discrete vascular strand and expression of *SCR* homolog around the latter, as seen in the single, centrally located vascular strand of the *Arabidopsis* root. In fact, this second type of endodermal structure encountered in *Equisetum* raises questions about the mechanisms controlling the radial patterning of tissues in those specimens (discussed earlier) that have a bi-endodermal structure (Fig. 1a). What is the identity of cells in the narrow strips encompassed by the two endodermal layers between neighboring vascular bundles? These cells must either produce SHR homolog themselves, or they must relay tangentially the SHR exported from vascular bundles. In either case they must then be exporting SHR centrifugally and centripetally to ensure the continuity of the two endodermal layers between neighboring vascular bundles. If these cells produce SHR homolog, then that would make them similar to vascular tissue. If they just relay SHR homolog, then they certainly do not express the *SCR* homolog, otherwise they would differentiate as endodermal cells themselves.

Back to one endodermal layer

The most puzzling endodermal feature of *Equisetum*, as seen through the perspective of an SHR-SCR positive feedback loop model, is, in fact, illustrated by those specimens that develop only one continuous endodermal layer, the outer endodermis (Fig. 1b). Given that these specimens have the same vascular architecture consisting of discrete bundles, and assuming that SHR/*SCR* homologs are generally exported/expressed in all directions around vascular tissue, the development of an outer endodermis alone implies alterations to the basic model. This could mean suppression of SHR export toward the nonvascular center of the stem or lack of SCR synthesis to the inside of the ring of bundles, or both. Similar situations, involving a hollow cylinder of vascular tissue (with or without leaf gaps) with what looks like ground tissue at the center and provided only with an outer endodermis (Fig. 1f), have been documented in several ferns in the Ophioglossaceae, Osmundaceae, and Vittariaceae (Ogura, 1972).

A possible explanation for the structure of all these pteridophyte stems that have only an outer endodermis, despite the fact that their vascular tissues do not go all the way to the center, could come from monocot roots. Some monocot roots are described as having a central pith, but a quick look at their development shows that the parenchymatous tissue that forms a central column is derived from procambium (Esau, 1977). Therefore, these apparently siphonostelic roots actually have cryptic protosteles, and, although it consists of parenchyma, their 'pith' has vascular tissue identity. As such, the 'pith' expresses genes characteristic of vascular tissue (or procambial) identity, such as an *SHR* homolog, and does not express the *SCR* homolog which is expressed only outside the ring of vascular tissue (Fig. 1f), as shown by Cui *et al.* (2007) for the rice root. Could it be that something similar happens in fern and horsetail stems with only an outer endodermis? Perhaps the proteome of cells in the nonvascular center of those stems somehow specifies them more as procambial cells than anything else. This explanation cannot be ruled out entirely before it is thoroughly tested – so there is much more to do in the study of molecular controls on development in seed-free plants, a rarely approached direction of plant biology.

A universal motif for specification of boundary layers

Accumulating evidence suggests that the SHR-SCR feedback loop mechanism could represent a universal motif responsible for the specification of a boundary layer around vascular tissues throughout the plant body, whether that layer is a functional endodermis (i.e. with suberized Casparian strips, as seen in roots and some stems), a more or less well defined starch sheath (in other stems; Esau, 1977), or a vascular bundle sheath (in leaves). Such evidence comes from studies in *Arabidopsis*, which have shown that SHR and SCR are required for the formation of a normal boundary layer in stems (Fukaki *et al.*, 1998), and that SCR is expressed in the layer immediately adjacent to the vascular tissues in stems, as well as in the bundle sheath cells of all veins (Wysocka-Diller *et al.*, 2000). If real, the universality of the SHR-SCR feedback loop mechanism in specifying boundary layers throughout the plant body and in all plants raises two important issues. One of these issues is that if the mechanism is common to all boundary layers, then specification of a functional endodermis would require the participation of additional mechanisms downstream of the SHR-SCR feedback loop.

The other issue has to do with the position of the boundary layer in dicot stems where this layer goes around the stele only to the outside of the vascular bundles. This is not what we would expect to see based on the distribution of boundary layers in nonprotostelic fern stems (as discussed earlier) and *Equisetum*. Seed plant stems have eusteles characterized by a ring of discrete vascular bundles arranged around a central pith. If specification of the boundary layer in these stems is controlled by the same SHR-SCR feedback loop mechanism, then we would expect to find this boundary layer forming individual sleeves either around each vascular bundle of the eustele (as in the *Equisetum* stems with individual endodermises around the vascular bundles), or on both sides of the ring of vascular bundles (as in bi-endodermal *Equisetum* and fern stems). Instead, dicot stems are more like those *Equisetum* stems provided with only an outer endodermis, and like the monocot roots with 'pith'. However, unlike the latter, the pith of dicot stems does not have vascular tissue identity so it would be interesting to know whether it expresses *SHR* even in the absence of vascular tissue identity. If so, that would represent additional evidence for the universality of the SHR-SCR feedback loop mechanism, but it would also intimate a certain

degree of uncoupling between *SHR* expression and vascular tissue identity.

Exaptation?

Going back to the initial hypothesis of a putative mechanism of endodermal specification conserved across all vascular plants, we can ask the following: what were the molecular origins in the evolution of such a mechanism? *SHR* and *SCR* homologs have been characterized in the gametophyte phase of the moss, *Physcomitrella patens* (Kitagawa *et al.*, 2006, 2007). However, moss gametophytes lack an endodermis and the functions of the two genes in *Physcomitrella* are unknown at present. Is it possible that *SHR* and *SCR*, conserved across embryophytes, illustrate the exaptation (a more elegant term for recruitment) of genes to new functions, during plant evolution, between the gametophyte and sporophyte phases, in the transition from the bryophytic condition to the polysporangiophyte-tracheophyte body plan? Shubin & Marshall (2000) have advocated a view of different organisms as mosaics of similar genes redeployed in different functional contexts during the evolution of gene regulatory interactions. Indeed, exaptation of function of existing genes (rather than evolution of new genes) at the transition between gametophyte-dominated and sporophyte-dominated life cycles, in embryophytes, is gaining increasing support from comparative genomic studies, which suggest that most of the gene families involved in developmental patterning in angiosperms are also found in mosses (Bowman *et al.*, 2007). An instance of exaptation of a molecular pathway from mosses to vascular plants is illustrated by the homologous *PpRSL1* and *AtRHD6* genes, which control, respectively, development of gametophyte rhizoids in *Physcomitrella* and root hair development in the *Arabidopsis* sporophyte (Menand *et al.*, 2007). As pointed out by Menand *et al.* (2007), such evolutionary transitions could be at the origin of the morphological radiation of the sporophyte phase early in the evolutionary history of vascular plants.

And back to *Equisetum*

Finally, not only is the type of endodermal structure a species-diagnostic character in *Equisetum* (Ogura, 1972, provides the most comprehensive, although not exhaustive, summary of the taxonomic distribution of the three endodermal structure types), but species of this genus present us with the very few documented examples of transition from one type of endodermal structure to another along the same plant. As documented by Bierhorst (1971) and discussed by Ogura (1972), the underground rhizomes and the bases of aerial stems of 12 *Equisetum* species (among them *E. hyemale*, *E. kansanum*, *E. ramosissimum*, *E. myriochaetum*, *E. laevigatum*, *E. robustum*) feature individual endodermal layers around each bundle, whereas, distally in the same plants, the aerial stems have an inner and an outer endodermis. Similarly, whereas the rhizomes and bases of aerial stems of *E. sylvaticum* exhibit an inner and an outer endodermis, aerial stems are characterized distally by a single outer endodermis. Interestingly, in all documented cases, the basal, underground part of the vertical stem has the same endodermal structure as the horizontal rhizome, and the transition to the endodermal structure of the aerial portion occurs at or near ground level (Bierhorst, 1971). The general pattern of these transitions between endodermal structure types in *Equisetum* seems to reflect a relaxation of the endodermal control on apoplastic transport from the below-ground to the above-ground parts of the plants. These structural transitions indicate that, whether it is a conserved SHR-SCR feedback loop mechanism or not, the mechanism that regulates endodermal specification in *Equisetum* is under environmental control. Thus, if SHR/SCR homologs were identified in *Equisetum*, this genus could provide a useful model system for understanding environmental controls on gene expression.

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Key words: *Arabidopsis*, endodermis, *Equisetum*, exaptation, ferns, monocots, root, SCARECROW, SHORTROOT.

Meetings

Automated soil respiration measurements: new information, opportunities and challenges

Automated Soil Respiration Workshop – a Terrestrial Ecosystem Response to Atmospheric and Climate Change (TERACC) sponsored workshop, Durham, New Hampshire, USA, September 2007

Soils are the largest carbon pool in terrestrial ecosystems, and soil respiration is the major pathway of carbon transfer from soil to the atmosphere. Measuring and predicting soil respiration has been challenging because the CO_2 efflux from soil integrates several complex below-ground processes (Zhou & Luo, 2006). Current models of soil respiration lack a theoretical underpinning with which to predict how fluxes reflect different plant and microbial CO_2 sources and mechanisms of CO_2 production. Recent technological advances in automated soil respiration (ASR) systems are generating unprecedented numbers of high temporal-resolution observations (Savage & Davidson, 2003). Automated soil respiration provides valuable information that is often missed with less frequent manual measurements, and presents the opportunity to move beyond empirical (gap-filling) models towards a predictive understanding of the key mechanisms that determine soil respiration fluxes. However, these continuous measurements present new challenges in that they require the additional management of complex equipment and large data sets as well as novel analytical approaches.

In September 2007, researchers met in Durham, New Hampshire, USA, for a workshop on ASR measurements (http://www.umaine.edu/teracc). The overall goal of the workshop was to initiate communication within the ASR measurement community and to provide a foundation for future research and syntheses studies. The meeting focused on: how automated measurements are advancing our understanding of soil respiration processes; challenges for the quality analysis (QA) and quality control (QC) of large data sets; and identifying current knowledge gaps and future research direction on soil respiration. The following questions provided the structure for the workshop.

- What are we learning from automated measurements of soil respiration?
- How do we know when we are making good measurements: QA and QC of large data sets?
- What are the best ways to analyze and model ASR data?
- What are the future research directions for automated chamber measurement studies and syntheses?

'... fast-changing diel patterns and dynamic responses to environmental events like precipitation pulses require ASR to quantify short-term responses.'

New insights from automated soil respiration measurements

Automated soil respiration techniques provide reliable and continuous measurements that can be deployed in complex terrain, near eddy covariance towers and at manipulation experiments. Several research groups and networks have begun ASR measurements that are starting to provide a solid basis for site-to-site intercomparisons (e.g. Ameriflux, Asiaflux, Carboeurope), and these efforts will be crucial for interannual comparisons across sites in the years to come.

