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Uncovering gene regulatory networks controlling plant cell differentiation

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Abstract

The development of multicellular organisms relies on the precise regulation of cellular differentiation. As such, there has been significant effort invested to understand the process through which an immature cell undergoes differentiation. In this review, we highlight key discoveries and advances that have contributed to our understanding of the transcriptional networks underlying *Arabidopsis* root endodermal differentiation. To conclude, we propose perspectives on how advances in molecular biology, microscopy and nucleotide sequencing will provide the tools to test the biological significance of these gene regulatory networks.

Keywords

cell differentiation; *Arabidopsis* root; endodermis

Mapping Gene Regulatory Networks to Understand Differentiation

Research across developmental systems has demonstrated that the molecular path from stem cell to differentiation is vastly complex [1]. A way to manage molecular complexity is to delineate the molecular inputs feeding into a process (e.g. differentiation), known as a gene regulatory network (GRN). The delineation of GRN architecture will generate hypotheses to address specific mechanisms of differentiation.

Here, we focus on how GRNs have been used to provide insight into differentiation of the *Arabidopsis* root endodermis, one cell type of the root ground tissue that has been the subject of intensive study. The cell types of the root are organized in radially symmetric cylinders around the inner, bilaterally arranged root vasculature (referred to as the stele in this review). The endodermis surrounds the stele and is often likened to a mammalian epithelium - it is polarized and plays the physiological role of a barrier to the external environment [2], reviewed in [3]. Since plant cells are immobile, entire cell lineages and developmental stages are easily traced along the longitudinal axis of the root. Stem cells and

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their rapidly dividing progenitors are located at the root tip (meristem) and give rise to all the mature tissue in the shootward portions of the root (Fig 1). These features make the Arabidopsis root ideal for developmental studies, and have enabled the rapid exploration of GRNs underlying cell differentiation in plants.

Recent work has expanded the boundaries of the endodermal GRN. First, we discuss recent contributions to our understanding of the GRN regulating two distinct asymmetric cell divisions that occur on the path to endodermis differentiation. This section reviews newly identified genes in the network, as well as how protein mobility affects GRN structure during these asymmetric cell divisions. We then highlight recent studies that link the early endodermal GRN to the cell biological features of endodermal differentiation. Finally, we discuss future perspectives highlighting emerging tools and technologies that can be applied to test the hypotheses generated based on knowledge of the endodermal differentiation GRN.

Gene regulatory networks in asymmetric cell divisions

The creation, generation, and expansion of the ground tissue relies on asymmetric cell divisions. These divisions are defined as asymmetric because the two daughter cells acquire different cell fates after division. There is no known factor that is inherited by one daughter cell and not the other, thus the daughter cells could acquire different fates after division or there is polarity in the mother cell that has yet to be appreciated [4]. In this section, we discuss the GRNs underlying two asymmetric cell divisions in the endodermal differentiation trajectory (Fig 1; Fig 2, Key Figure). The first asymmetric division generates the precursors of the endodermis and the adjacent cortex cell lineage. The second asymmetric division of the endodermis occurs in older seedlings and forms an additional layer of ground tissue called middle cortex.

Asymmetric division of the cortex/endodermis stem cell relies on feed forward loops

The endodermis originates from an asymmetric cell division of a progenitor within the meristem at the tip of the root [5]. The progenitor cell is called the cortex-endodermal initial (CEI) and undergoes a first asymmetric division to regenerate itself and give rise to the CEI daughter cell (CEID). The CEID then undergoes a second asymmetric division to generate the progenitors of the endodermis and the cortex (Fig 1A, 2A). The GRN regulating this second stem cell asymmetric division has been studied for nearly two decades and provides a model for understanding fundamental mechanisms in stem cell division.

At the core of the GRN regulating asymmetric division of the CEID are two plant-specific GRAS-family transcription factors (TFs), SHORTROOT (SHR) and SCARECROW (SCR) [6–9]. In mutant plants lacking either gene, the CEID fails to undergo asymmetric cell division, resulting in a single mutant layer of ground tissue [6,8,9]. SHR activates expression of *SCR*, then forms a complex with SCR protein to regulate a common set of downstream target genes [9–11]. SHR is a mobile TF that is transcribed and translated in the stele and then moves into the adjacent cell layers through plasmodesmata [12,13]. In the adjacent CEID cells, the SHR-SCR complex generates a feedback loop to upregulate expression of

SCR, which leads to the asymmetric cell division [11,14]. This is facilitated by SHR-SCR activation of a D-type cyclin, *CYCD6*, which in turn disrupts the sequestration of SCR by RETINO-BLASTOMA-RELATED (RBR) protein in the nucleus, freeing more SCR to complex with SHR resulting in a positive feedback loop (Fig 2A) [15,16]. Mathematical models of these GRN motifs predict this architecture has an important role in establishing the dynamics that results in the asymmetric cell division of the CEID [16]. While these factors are critical to ground tissue establishment, the nature of their interaction with DNA and other transcriptional regulators is still not fully understood.

Identifying new nodes in the GRN underlying asymmetric cell division of the CEID

Recently, several members of the indeterminate domain (IDD) TF family have been identified as having a role in regulating CEID division [17]. No single mutant in an IDD gene has a severe endodermal differentiation phenotype, although *jackdaw* mutants have an additional layer of ground tissue [18]. In higher order IDD mutants, the ground tissue undergoes abnormal divisions with both increased and reduced numbers of cell layers within the same root [17,18]. Strikingly, a triple mutant (*bluejay*, *jackdaw* and *scr*) eliminates ground tissue identity entirely, implying that the IDD family also plays a role in specifying the ground tissue [17].

In gibberellic acid (GA) signaling, IDD proteins mediate interaction between DNA and GRAS-family TFs [19]. Work from several groups has demonstrated that this is similar for SHR/SCR and IDD TF interactions. Yeast-2-hybrid experiments have shown that SHR and SCR can bind to JACKDAW, as well as to its closest homologs BALD IBIS, and MAGPIE [18,20]. Moreover, a recent manuscript describes the crystal structure of the SHR-SCR-JKD and SHR-SCR-MGP complexes [21]. These results indicate that SHR-SCR cannot bind to DNA alone, but require formation of a complex with an IDD TF.

Additional GRAS-family TFs also function in endodermal specification. For example, the closest SCR homolog, SCARECROW-LIKE 23 (SCL23) is expressed mainly in the endodermis [22]. The *scl23* single mutant has no apparent endodermal differentiation defects, but the *scl23;scr* double mutant is more severe than *scr* alone and completely lacks features associated with endodermal differentiation [22]. Aside from TFs, other types of proteins have been shown to have key roles in regulating the CEID division. Yeast-2-hybrid assays have identified the SHR-interacting vacuolar trafficking protein, SHRUBBY (SHBY), as a regulator of the CEID division [23]. In *shby* mutants, there are additional ground tissue divisions, even in *scr* or *shr* mutant backgrounds, resulting in extra cell layers with cortical cell identity [23].

The GRN regulating the middle cortex formation recycles nodes in the CEID GRN

In *Arabidopsis*, endodermal progenitors of the root meristem undergo another asymmetric cell division in 7–10 day old seedlings [24]. These divisions give rise to an additional cortex

layer called middle cortex [25] (Fig 1B, 2B). SHR, SCR and another GRAS-family TF, SCL3 all play a role in middle cortex formation and the SHR-SCR complex can bind and regulate expression of SCL3 [10,11]. In a *shr* mutant there is neither endodermis nor middle cortex [25]. High levels of SHR in the endodermis result in the formative stem cell division giving rise to cortical and endodermal progenitors, while medium levels induce middle cortex divisions [26]. Thus, SHR itself has a dosage-dependent effect on the asymmetric cell division resulting in middle cortex formation [26]. Both SCR and SCL3 act to repress middle cortex division, as single mutants of either results in precocious middle cortex formation [25,27]. Recently, a predicted transcriptional co-regulator, SEUSS (SEU), was identified as a positive regulator of *SHR*, *SCR* and *SCL3*, and prevents middle cortex formation by keeping *SHR* levels high [28]. Although SEU has been shown to be a negative co-regulator in flower development, in middle cortex formation it can directly bind to upstream regions of *SHR*, *SCR* and *SCL3* [28–30]. This suggests the role of SEU is context dependent and characterizing its interaction factors and its binding to DNA will be of interest for further study.

GA signaling plays a major role in regulating middle cortex formation [25], and the extent to which GA and SHR/SCR function together is still under investigation. *shr* mutant plants are insensitive to GA inhibitors and *scr* mutants treated with GA inhibitors have additive effects on precocious MC formation [25]. *SCL3* is regulated by SHR/SCR and promotes GA signaling, acting as a convergent point for these two pathways in the endodermis [31]. Like *shr* mutants, *shby* is insensitive to effects of GA inhibitors on middle cortex formation, suggesting that SHBY is required for middle cortex regulation via GA signaling [23]. Recently, it has been shown that GA represses *SCL3* indirectly by repressing the *SCL3*-activator SEU, resulting in precocious middle cortex formation [28]. This provides another common link between the SHR/SCR pathway and the GA pathway in middle cortex formation [28]. It is notable that *CYCD6* is expressed at the onset of middle cortex formation and that *seuss* and *shby* mutants, as well as wild-type roots, treated with GA inhibitor have expanded *CYCD6* expression [23,28]. Therefore, it is likely that GA and SHR/SCR also commonly regulate *CYCD6*.

SHR regulation by sequestration and establishment of expression domain

The regulated movement of SHR from the stele into the CEID is critical to initiate asymmetric cell division and commence the endodermal differentiation program [12]. Differences in the number of ground tissue layers that form across plant species is regulated by the mobility of SHR through the ground tissue [32]. There have been many conceptual advances regarding the cell biology regulating SHR mobility, which was recently reviewed [33]. Here, we focus on the TFs that have dual roles in specifying the endodermis and restricting SHR movement.

SHR is localized to both the cytoplasm and nucleus in the stele, but it is localized strictly to the nucleus in the endodermis [12] and must be present in both the cytoplasm and nucleus in the stele in order to move between cell layers [34,35]. One simple way to limit SHR movement beyond the endodermis is to sequester SHR into the nucleus. Several proteins critical to the asymmetric division GRN also play a role in sequestering SHR in the nuclei of

the endodermis. A *SCR-RNAi* line that depletes *SCR* expression, but retains two ground tissue layers, results in SHR movement beyond the endodermis into the cortex. Moreover, ectopic expression of *SCR* in the stele sequesters SHR into the nucleus and prevents further movement [11,14]. Nuclear sequestration of SHR within the endodermis is also regulated by interaction with IDD TFs [18,20]. This relationship is inferred by ectopic expression of *JACKDAW* and *MAGPIE* in the stele leading to sequestration of SHR into the nucleus [20]. *SCL23* also has a role in regulating SHR movement. When *SCL23* is ectopically expressed in the stele, SHR does not move to the neighboring layer although the nuclear-cytoplasmic partitioning is unchanged, implying *SCL23* affects SHR movement through a mechanism other than nuclear sequestration [22].

SHR mobility is regulated at multiple levels, which is not surprising since SHR is broadly potent for specifying ground tissue fate and is considered a “master regulator” of endodermal identity [9,36]. Thus, the regulation of SHR expression itself is also likely to be tightly regulated. The question of how transcriptional cascades are initiated was recently addressed for the SHR-SCR pathway through utilization of yeast-1-hybrid assays. In this manuscript, the authors generated and tested a model that suggests the combinatorial regulation of multiple activators and repressors functioning in opposing cellular domains is required to establish *SHR* expression in a specific domain [37]. This study provides one explanation as to how expression can be established and maintained in the absence of *a priori* specificity. This mechanism contrasts with how the other “master regulator” of endodermal identity, *SCR*, expression is established, which is mainly via activation by one factor (SHR) followed by positive transcriptional feedback on itself [37].

Future perspectives in the CEID and middle cortex GRNs

While these studies have shed light on the GRN regulating asymmetric cell divisions in the meristem, many questions remain unanswered. The IDD TFs have roles in both division and SHR sequestration. While it has been shown that SHR-SCR can bind to *JACKDAW* and *MAGPIE* (and may be required to do so for SHR-SCR DNA binding function), the expression domains of *JACKDAW* and *MAGPIE* are restricted to immature meristem tissue while *SHR* and *SCR* are expressed throughout the root endodermis [38,39]. It will be interesting to determine if SHR-SCR can also form a complex with IDDs that are expressed in the more mature parts of the root [17]. Given the variety of phenotypes in the higher order IDD mutants, it is also possible that, like SHR and SCR, IDDs play a role in the middle cortex division GRN. Traditional reverse genetics in combination with assays in heterologous systems, like yeast-1- and yeast-2-hybrid assays might provide the means to identify new nodes in the endodermal GRN. Finally, the relationships among SHR’s cellular localization, mobility, and transcriptional function are still enigmatic. While we know which SHR protein domains are necessary for movement and that there are several mechanisms in place to sequester SHR into the endodermal nucleus, how important nuclear sequestration is to SHR transcriptional output is still undetermined.

Expanding the endodermal GRN to encompass differentiation

As cells exit from the meristematic zone along the root longitudinal axis, they undergo elongation and then acquire specialized features. Endodermis differentiation is marked by the presence of centrally positioned, ring-like cell wall impregnations called the Casparian Strip (CS) (Fig 1C). The CS is a water impermeable structure composed of lignin, which has important physiological roles for ion uptake and hydration [40]. Beyond the CS, endodermal cells make suberin, a waxy, protective cell wall component. This suberization is referred to as a “second differentiation” and occurs sporadically in endodermal cells [41].

Unlike endodermal divisions in the meristem, the mechanisms underlying development of the CS were largely unknown until recently. While it has been known for over a decade that the CS is completely missing in *shr* mutants and not fully formed in *scr* mutants, it was only recently that new nodes in this network have been identified [6]. The CS domain is highlighted by a family of transmembrane proteins collectively called Casparian Strip Associated Proteins (CASPs), which localize to the outer periphery of endodermal cells and have been proposed to serve as a guide for lignin synthesis and assembly [42]. Since this discovery, a number of forward genetic screens have enabled the identification of endodermal differentiation regulators, as marked by CS function and CASP localization. In this section, we review the recent expansion of genes downstream of SHR and SCR that regulate endodermal differentiation, especially highlighting TFs and receptor-like kinases. While the secondary differentiation through suberin deposition is less understood than CS development, novel insights into the relationship between suberization and nutrient uptake provide initial clues to understanding the transcriptional events underlying this second differentiation.

MYB36 links specification to differentiation

The TF MYB36 was identified in multiple forward genetic screens as a key regulator of CS formation [43,44] (Fig 2C). MYB36 is required for *CASP* expression and up-regulates other CS-related genes, such as peroxidases that polymerize monolignol subunits, as well as other known lignin-assembly proteins [43–46]. Moreover, MYB36 can directly bind to the upstream regulatory regions of *CASPs* and other CS-related genes [43], though this interaction likely requires a co-factor, which is also downstream of MYB36 [44]. Despite lacking a functional CS, *myb36* mutants display a remarkably normal developmental phenotype with minor differences in root growth rate and length [43,44,47]. While the formation of CS is delayed, a barrier is eventually formed in more mature tissue [44] and there is overproduction of endodermal suberin [43]. Aside from this, the only other morphological phenotype of *myb36* mutants is premature middle cortex formation [44,48].

Like SCR, MYB36 is specifically expressed in the endodermis, although it is more strongly expressed in differentiating cells than meristematic cells [39,43,44,49]. Given its expression pattern and role in CS formation, it is unsurprising that *MYB36* was identified as a target of SHR-SCR in time course induction experiments [15,44]. This makes MYB36 a key transcriptional link between SHR-SCR-mediated specification and CS-marked differentiation [44]. Interestingly, MYB36 can negatively feedback on the expression of the IDD TFs, although the directness of feedback remains to be elucidated [44]. One proposed

hypothesis is that premature middle cortex formation in the *myb36* mutant is the result of increased expression of IDD TFs in the *myb36* background [44]. Together these results suggest that specification and differentiation are tightly linked processes and there is feedback regulation between each process.

The cell biological components of the endodermal differentiation GRN

Forward genetic screens for defects in CS localization and function have identified two receptor-like kinases, SCHENGEN1 (SGN1) and SCHENGEN3 (SGN3), which are enriched in the endodermis [50,51]. In *sgn1* mutants, CASP proteins do not align along the central plane of endodermal cells, and a functional CS barrier is not formed [50]. Similarly, *sgn3* mutants lack a functional barrier, however this is due to interruptions in CS domains, presenting a “beads on a string”-like expression of CASPs [51]. This pattern can also be observed in wild-type roots in the first endodermal cells of the differentiation zone [45,51]. In a wild-type situation, these immature domains eventually seal completely to form a barrier. This implies that, while *sgn1* and *sgn3* mutants have an immature endodermal cell lineage, they lack the differentiation signaling to form a CS.

Both SGN1 and SGN3 are expressed in polar domains along the plasma membrane. SGN1 is localized to the membrane facing the cortex (outer) and SGN3 is confined to the inner CS domain plane along the cell periphery in mature endodermal cells with a CS [50,51]. Interestingly, *SGN1* expression is diminished in a *myb36* mutant while *SGN3* is relatively unaffected, suggesting that different mechanisms exist for regulating expression of these key receptors [50]. Recently, two complementary efforts uncovered the existence of signaling peptides CASPARIAN STRIP INTERGRATING FACTOR 1 and 2 (CIF1 and CIF2) that act as the ligand for SGN3 to promote CS formation [52,53]. CIF1/2 are produced in the stele, suggesting that CIF1/2 might move from the stele prior to complete CS formation, thereby serving as a SGN3-dependent signal to the endodermis that the root barrier is not yet complete [52,53]. There is some overlap in SGN1 and SGN3 expression domains and epistasis experiments suggest that the SGN1 and SGN3 receptors function together in some, but not all contexts [50,52]. The defects in CASP expression are more severe in a *sgn1 sgn3* double mutant than in either alone, indicating some independence of their function [50], however, the enhanced suberization upon CIF treatment of wild-type plants is decreased in a *sgn1* mutant background, supporting that SGN1 can propagate CIF-activated signals independently from SGN3.

Future perspectives in the endodermal differentiation GRN

It is still unknown how extensively these processes are connected to earlier transcriptional specification events. MYB36 provides one direct link between SHR-SCR and the expression of CASPs, peroxidases and SGN1 [43,44]. It will be interesting to see if other stele-derived factors, like SHR and CIF1/2 emerge as a common theme by which non-autonomous signals regulate endodermal development.

In comparison to CS formation, the mechanisms underlying the specification of suberin are largely undiscovered. Recent work has demonstrated the endodermis can regulate

suberization depending on nutrient availability and that different nutrients can affect suberization in opposing ways [54]. Interestingly, none of the nutrients tested affected CS formation, implying that distinct GRNs control the formation of these specialized features [54]. The components of the suberin-specifying GRN may be downstream of hormone signaling since suberin deposition in response to nutrient availability is conveyed through opposing effects of abscisic acid (ABA) and ethylene signaling [54]. It will be important to identify the key transcriptional components that control suberization, and how these components are connected to earlier endodermal specification events.

Lastly, there appears to be feedback between CS formation and suberization [46]. Many CS mutants, including *sgn1* and *myb36*, produce an overabundance of suberin [43,50]. Furthermore, CIF2 can induce precocious suberization in a SGN3-dependent manner [52]. While *sgn3* has no defects in suberin, double *sgn3 sgn1* mutants suppress the overabundance of suberin seen in *sgn1* alone [52]. Together, these results imply a role for these receptors in sensing the levels of suberization and highlight the complexity of feedback among these signals. Identification of transcriptional links between these processes will certainly break ground, furthering our understanding of differentiation.

Emerging tools to test the significance of GRN architecture

Elucidating the components and interactions in the endodermal GRN has been a major undertaking. Looking ahead, we predict there will be a switch from defining the GRN players and their relationships to testing hypotheses that probe our understanding of the functional outcome of these relationships.

Advances in imaging techniques such as fluorescence correlation spectroscopy (FCS), can be employed to answer questions about protein mobility and complex formation [55,56]. In the CEID division GRN, the mobility of SHR and interaction with SCR are required for proper endodermis formation. However, until recently, measurement of the mobility of SHR or the dynamics of its interaction with SCR was not feasible. Recently, Clark et al. pioneered the use of FCS in plants to measure the diffusion coefficient of SHR in the stele and endodermis, and employed this technique to determine the stoichiometry of the SHR-SCR complex [57]. These measured values were integrated into a previous mathematical model of SHR-SCR dynamics, which did not account for the intercellular mobility of SHR or the oligomeric state of the SHR-SCR complex [16,57]. Using measured parameters, a simulation of the SCR-SHR complex takes 18–24 hours to reach steady-state and shows that the model is sensitive to the diffusion rate of SHR [57]. These results underscore the role of diffusion in the temporal outputs of GRNs and demonstrate how FCS can improve existing models. FCS might further provide insight into complex formation and the oligomeric state of the SHR-SCR-IDD complexes during CEID division, middle cortex formation, and other developmental stages of the root.

While differentiation is traditionally viewed as unidirectional and uniform, recent developments from single cell RNAseq analyses suggest that the differentiation trajectory is probabilistic [58,59]. Improvements in single cell analyses could be employed to analyze stochasticity and robustness of GRNs. One key finding in single cell RNAseq-based transcriptional analysis from *Arabidopsis* is that cells from a seemingly uniform population

can have wide variance in expression across hundreds of gene, even when accounting for technical noise [60]. A similar approach could be used to determine how much levels of key TFs like SHR, SCR and MYB36 fluctuate within an endodermal cell population. Further, single-cell sequencing approaches can be complemented with advances in live-imaging that use light sheet microscopy to quantify levels of fluorescently-labeled TFs in individual cells. Light sheet microscopy has the added advantage of imaging roots in their native orientation for long periods of time with reduced phototoxicity compared to traditional confocal set ups [61,62]. Beyond GRN robustness, single cell analyses can glean insight into cell states unappreciated by analysis of bulk populations [60]. Bioinformatics tools have been developed to order single cell RNAseq data along a longitudinal differentiation trajectory in *Arabidopsis* roots [63,64]. This provides a means to analyze single cell RNAseq data and to identify a developmental or cell identity state [65]. These tools could be especially interesting to apply to IDD mutants that have either superfluous and reduced layers of ground tissue to determine if cells in mutant layers more closely resemble endodermis or cortex. Ultimately, a combination of sequencing and imaging approaches will shed light on hypotheses that posit differentiation has more flexibility to proceed in more directions than previously appreciated.

Concluding Remarks

The root endodermal GRN is perhaps one of the best-characterized GRNs in plant development, yet much remains to be discovered. The GRN underlying early stem cell divisions is mapped precisely enough that it is possible to test and predict how TF diffusion and interactions affect division output and timing. Beyond the meristem is a frontier of undiscovered transcriptional events that lead to production and localization of specialized features required for endodermal physiology. Developing technologies, such as FCS, single cell RNAseq and light sheet microscopy, along with tested methods, such as forward genetic screens, will be important for understanding the cascade of transcriptional events underlying cell fate decisions. Another useful approach to understand endodermis differentiation is a “bottom up” approach, which introduces network components into a naïve system to determine which are fundamental for endodermis differentiation. Our understanding of the GRN underlying stem cell division and differentiation in the endodermis will lay the groundwork for comparative genomics of other cell fate processes.

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GLOSSARY

Asymmetric cell division

A cell division in which the two resulting daughter cells acquire different cell fates

Casparian Strip

The ring-like cell wall impregnations made of lignin, specific to the root endodermis

Cortex

The cell layer(s) surrounding the root endodermis

Endodermis

The inner cell layer of the root surrounding a vascular bundle

Fluorescence Correlation Spectroscopy

an analysis of the correlation of fluctuations of fluorescent intensity used to measure parameters of molecular mobility, direction and stoichiometry

Gibberellic Acid

A plant hormone with many roles, including promoting growth

Ground Tissue

Refers to both the endodermis and cortex cell-types

Higher order mutant

A mutant defective in multiple genes of the same gene family, often used in cases where genes are thought to be functionally redundant

Lignin

A class of organic polymers deposited in cell walls

Meristem

A region of rapidly dividing cells and stem cells in multi-cellular plants

Plasmodesmata

Cytoplasmic channels containing endoplasmic reticulum that connect neighboring plant cells

Stele

Root vascular bundle and pericycle cell-types

Suberin

A waxy, impermeable substance present in cell walls

Yeast-1-hybrid

A transcription factor-DNA binding assay in yeast based on activation of a reporter gene

Yeast-2-hybrid

A protein-protein binding assay in yeast based on activation based on activation of a reporter gene

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Outstanding Questions

- Do IDD_s expressed in mature endodermis tissue form a complex with SHR-SCR? If so, what kinds of genes does the SHR-SCR-IDD complex regulate in mature tissue? If not, what are the SHR-SCR binding partners in the mature tissue?
- How do the IDD_s fit in the middle cortex division GRN? Does MYB36 regulate middle cortex formation through mediating *IDD* expression?
- What are the TFs regulating the second differentiation of the endodermis, suberization? Are these related to earlier specification events and if so, how?
- How does CS formation and function signal to suberization (i.e. what is the underlying GRN)?

TRENDS Box

The endodermis is a model for plant cell differentiation that has a relatively well-mapped GRN from stem cell through differentiation (Casparian strip formation).

Recent studies based on forward and reverse genetic screening, yeast-1- and yeast-2-hybrids have identified many novel proteins in the endodermis differentiation, expanding our understanding of the GRN.

The IDD family of transcription factors plays a key role in the asymmetric stem cell division of the endodermis by regulating tissue specification and interacting with SHR-SCR.

Endodermis differentiation is linked to specification through transcription factor MYB36, which activates expression of Casparian strip-associated assembly genes.

Recent developments in microscopy technologies improve models of cell division dynamics by measuring transcription factor mobility and stoichiometry.

Figure 1. Endodermis Differentiation Along the Arabidopsis Root Longitudinal Axis. Schematic of the CEI and CEID in (A), middle cortex division in (B) and Casparian Strip formation in (C). Legend (D) indicates cell types and features.

Figure 2.

Gene Regulatory Networks in Endodermis Differentiation. (A) CEID asymmetric cell division GRN. Stele-derived TF *SHR* activates expression of *SCR*. *SCR* protein activates expression of itself and forms a complex with *SHR* and the *IDD* TFs. *SHR-SCR* promote *CYCD6* expression and *CYCD6* disrupts sequestration of *SCR* by *RBR* creating a positive feedback loop. High levels of *SHR-SCR* promote *CYCD6* and initiate asymmetric cell division. (B) Middle cortex division GRN. *SHR*, *SCR* and *SCL3* promote middle cortex division. *SEU* keeps levels of *SHR* high, preventing middle cortex formation in young seedlings. *SEU* is inhibited by *GA* signaling, reducing *SHR* to medium levels to promote middle cortex formation. *SCL3* is responsive to both *SHR-SCR* and *GA* signaling via *SEU* in preventing premature middle cortex formation. (C) Casparian strip development GRN. *SHR-SCR* activates *MYB36* expression, which binds upstream of Casparian Strip associated factors such as *CASP*s, peroxidase *PER64* and dirigent-domain containing protein *ESB1*. *MYB36* inhibits expression of *IDD*s and activates expression of *SGN1*. Stele-derived ligands *CIF1/2* binds and promotes activity of *SGN3*.