UC Davis

UC Davis Previously Published Works

Title

Omics and modelling approaches for understanding regulation of asymmetric cell divisions in arabidopsis and other angiosperm plants

Permalink

https://escholarship.org/uc/item/562521pm

Journal

Annals of Botany, 113(7)

ISSN

0305-7364

Authors

Kajala, Kaisa
Ramakrishna, Priya
Fisher, Adam
et al.

Publication Date

2014-06-01

DOI

10.1093/aob/mcu065

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Annals of Botany **113**: 1083–1105, 2014
doi:10.1093/aob/mcu065, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

INVITED REVIEW

Omics and modelling approaches for understanding regulation of asymmetric cell divisions in arabidopsis and other angiosperm plants

Kaisa Kajala[1], Priya Ramakrishna[2], Adam Fisher[3], Dominique C. Bergmann[4], Ive De Smet[2,5,6], Rosangela Sozzani[3], Dolf Weijers[7] and Siobhan M. Brady[1,*]

[1]*Department of Plant Biology and Genome Center, UC Davis, Davis, CA 95616, USA,* [2]*Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, UK,* [3]*Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC 27695, USA,* [4]*Howard Hughes Medical Institute and Department of Biology, Stanford University, Stanford, CA 94305, USA,* [5]*Department of Plant Systems Biology, VIB, Technologiepark 927, B-9052 Ghent, Belgium,* [6]*Department of Plant Biotechnology and Genetics, Ghent University, Technologiepark 927, B-9052 Ghent, Belgium and* [7]*Laboratory of Biochemistry, Wageningen University, Dreijenlaan 3, 6703HA Wageningen, The Netherlands*
** For correspondence. E-mail sbrady@ucdavis.edu*

Received: 27 December 2013 Returned for revision: 31 January 2014 Accepted: 6 March 2014 Published electronically: 13 May 2014

• *Background* Asymmetric cell divisions are formative divisions that generate daughter cells of distinct identity. These divisions are coordinated by either extrinsic ('niche-controlled') or intrinsic regulatory mechanisms and are fundamentally important in plant development.
• *Scope* This review describes how asymmetric cell divisions are regulated during development and in different cell types in both the root and the shoot of plants. It further highlights ways in which omics and modelling approaches have been used to elucidate these regulatory mechanisms. For example, the regulation of embryonic asymmetric divisions is described, including the first divisions of the zygote, formative vascular divisions and divisions that give rise to the root stem cell niche. Asymmetric divisions of the root cortex endodermis initial, pericycle cells that give rise to the lateral root primordium, procambium, cambium and stomatal cells are also discussed. Finally, a perspective is provided regarding the role of other hormones or regulatory molecules in asymmetric divisions, the presence of segregated determinants and the usefulness of modelling approaches in understanding network dynamics within these very special cells.
• *Conclusions* Asymmetric cell divisions define plant development. High-throughput genomic and modelling approaches can elucidate their regulation, which in turn could enable the engineering of plant traits such as stomatal density, lateral root development and wood formation.

Key words: Asymmetric cell division, embryo, root, stomata, lateral root, cortex endodermis initial, omics, *Arabidopsis thaliana*, *Zea mays*, maize.

INTRODUCTION

Asymmetric divisions are fundamentally important for plant morphogenesis

Asymmetric cell divisions are fundamental for multicellular organism development. The generation of daughter cells with distinct identities is referred to as the 'asymmetric' property of these cell divisions. In some cases, distinctly specified daughter cells are then the precursors for a cell type population that will proliferate, as will be described in the section on embryonic and lateral root divisions. In others, the initial cell retains its ability to proliferate, as will be described in the section on the cortex/endodermis initial and on procambium and cambium. In arabidopsis stomatal asymmetric divisions, however, there is further (though limited) proliferation of both the initial and the daughter cells. Asymmetric cell divisions in all plant species are considered to be formative because they establish axis and organ polarity, tissue patterning and morphogenesis. The orientation of asymmetric cell divisions can be periclinal (parallel to the plane of elongation) and act to regulate the overall shape and pattern of the plant (Robinson *et al*., 2011) or anticlinal (against the plane of elongation). In tissues such as the epidermis, where all cell divisions are anticlinal, division orientations relative to other landmarks are critical.

Looking to the inside: intrinsic asymmetric divisions

Asymmetry can be determined by either intrinsic or extrinsic mechanisms (summarized in Fig. 1). In intrinsic asymmetric cell divisions, there is unequal segregation of identity determinants within the cell. This unequal segregation can be accomplished by an intrinsic signal, including competitive segregation of determinants, or post-translational protein modifications. In most plant cells, a pre-prophase band marks the division site and placement of the cell plate during cytokinesis (Rasmussen *et al*., 2011). Consequently, to carry out an intrinsic asymmetric cell division in plants, the position of the pre-prophase band and hence the orientation of the cell division plane have to be regulated and coordinated with cell identity-determinant distribution.

© The Author 2014. Published by Oxford University Press on behalf of the Annals of Botany Company. All rights reserved.
For Permissions, please email: journals.permissions@oup.com

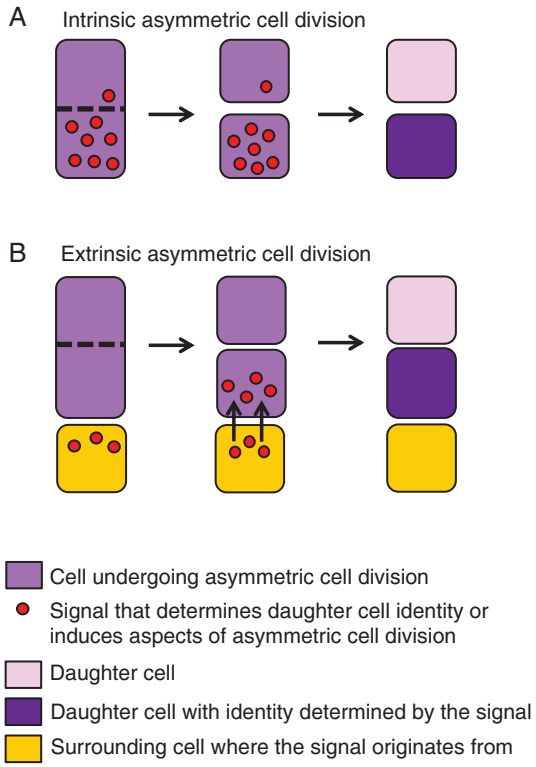

FIG. 1. Intrinsic and extrinsic asymmetric divisions. (A) In intrinsic asymmetric cell divisions the polarity of the division plane and the daughter cell identity are determined by the distribution of intrinsic determinants. (B) In extrinsic, or niche-controlled, asymmetric cell divisions the polarity and/or daughter cell identity are determined by the distribution of extrinsic signals, such as non-cell-autonomous transcription factors or signalling peptides from neighbouring cells. These schematic figures reflect the theoretical framework. The same colour palette is used in all figures to show the roles of the different players involved in asymmetric cell divisions.

The ultimate peer pressure: defining your identity based on external influence

Extrinsic asymmetric divisions are also referred to as 'niche-controlled' asymmetric cell divisions. Here, the division plane is oriented to position the daughter cells such that they will be located in distinct niches, or local environments, and exposed to different intercellular identity-determining signals. In some cases this extrinsic signal may be from a neighbouring cell that has secreted a signal or provides a biomechanical stimulus. Alternatively, the extrinsic signal may be a gradient of a signalling molecule across a field of cells.

Asymmetric divisions in plants versus animals

Despite asymmetric cell divisions being of fundamental importance in both plants and animals, development differs in key ways between plants and animals. Firstly, plant cells are fixed in position by the rigid extracellular cell wall (Cosgrove, 2005), leading to structural differences in cytokinesis compared with animals – specifically, the use of a contractile ring in animals versus wall building preceded by positioning of a pre-prophase band in plants (Rasmussen *et al*., 2011). Furthermore, these cell walls prevent cell migration, a central process in animal development (Lauffenburger and Horwitz, 1996). Secondly, the plant continues to initiate and establish new organs post-embryonically. While animals develop a predetermined number of limbs in the embryo, plants reiterate organogenesis. Asymmetric cell divisions play a crucial role in enabling post-embryonic organogenesis, for example in lateral root initiation (Malamy and Benfey, 1997; Malamy and Ryan, 2001; De Smet *et al*., 2008) and in lateral branching via the shoot apical meristem (Lee and Clark, 2013). Additionally, plants exhibit increased complexity in development as environmental cues feed into the regulation of asymmetric cell divisions that produce new organs (Zhang and Forde, 1998). Extrinsic signals are of particular importance to plants, since most plant cells are pluripotent and cell fate is largely under positional regulation (van den Berg *et al*., 1995, 1997; Reinhardt *et al*., 2003, 2005).

In order to generate two daughter cells of separate identities, molecules that regulate distinct identities must be deployed. Similar classes of molecules have been described in plants and in animals, including ligand–receptor pairs (Clark *et al*., 1997; Rojo *et al*., 2002, Tanaka *et al*., 2002; Fisher and Turner, 2007; Fuller and Spradling, 2007; Hirakawa *et al*., 2008; De Smet *et al*., 2008), signalling pathway components (Yu *et al*., 2003; Schweisguth, 2004; Le Borgne *et al*., 2005; Song *et al*., 2006), transcription factors (Spana and Doe, 1995; Nakajima *et al*., 2001; Schlereth *et al*., 2010) and microRNAs (Emery *et al*., 2003; Lu *et al*., 2008; Cordes *et al*., 2009; Zhao *et al*., 2009; Carlsbecker *et al*., 2010). While the classes of molecules are similar, the exact gene products identified to date are distinct between the kingdoms.

Asymmetric cell divisions in animal embryonic development that have been elucidated in the greatest mechanistic detail are intrinsic. This is exemplified by the behaviours of *Drosophila* neuronal precursors, which employ unequal division of cell identity determinants such as the Notch pathway repressor Numb, to yield sensory organ precursor and neuroblast cells (Schweisguth, 2004; Le Borgne *et al*., 2005), the transcription factor Prospero (Spana and Doe, 1995) and the inhibitor of ribosome biogenesis and cell growth Brat in *Drosophila* ganglion mother cells (Betschinger *et al*., 2006).

In animals, niche-controlled or extrinsic mechanisms can be important when cells are confined to a particular position. For example, human myeloid stem cell daughter identity is determined by extracellular haematopoietic growth factors (Clark and Kamen, 1987). Daughter cell identity in the *Drosophila* ovarian germ line is determined by unequal levels of the diffusible ligands Dpp and Gbb from surrounding cells (Fuller and Spradling, 2007). We will discuss in detail a range of plant developmental processes that are currently understood to be niche-controlled, namely embryo development, primary root initial cell divisions and differentiation, lateral root initiation and (pro)-cambial development. Furthermore, we will discuss stomatal development, as it is a unique example of a combination of intrinsic and niche-controlled identity determination.

Using omics and modelling approaches to characterize plant asymmetric cell divisions

In both plants and animals, asymmetric cell divisions occur in small cell populations that are not always experimentally tractable, and their regulation likely requires complex, dynamic

interactions. Traditional approaches to studying asymmetric cell divisions have involved mutagenesis and screening for altered fate or division phenotypes, and many core regulatory factors have been identified in this fashion (Benfey *et al*., 1993; Scheres *et al*., 1995; Laux *et al*., 1996; Fisher and Turner, 2007; MacAlister *et al*., 2007; Dong *et al*., 2009). However, these forward genetics approaches are limited in their depth. Furthermore, interfering with asymmetric cell divisions that occur early during embryogenesis can cause embryo lethality. Recent advances in 'omic' approaches can lend themselves to deepening our understanding of these regulatory interactions. For example, cell type-specific gene expression and chromatin modification studies (Brady *et al*., 2007; Mustroph *et al*., 2009; Deal and Henikoff, 2010; Sozzani *et al*., 2010; Pillitteri *et al*., 2011), DNA–protein interactions (Levesque *et al*., 2006; Gaudinier *et al*., 2011) and high-throughput phenotypic screening (Bruex *et al*., 2012; Kang *et al*., 2013; Sankar *et al*., 2014) can be combined with computing tools such as co-expression analyses (Brady *et al*., 2007; Sozzani *et al*., 2010), network analysis (Bruex *et al*., 2012) and modelling (Savage *et al*., 2008; Robinson *et al*., 2011; Cruz-Ramirez *et al*., 2012). For each of the developmental processes reviewed, we will discuss how these approaches have benefited their elucidation.

ZYGOTE AND EMBRYO POLARITY

At the beginning: asymmetric divisions in the embryo

Plant embryogenesis is the first morphogenetic phase of life, and it generates the precursors of all major tissues, as well as the stem cells that maintain these tissues post-embryonically (Weigel and Jurgens, 2002). While in some plants divisions appear chaotic, at least at the histological level (Johri *et al*., 1992), other species, including arabidopsis, have highly regular division patterns (Fig. 2A) (Jürgens and Mayer, 1994). This is one of the reasons why arabidopsis (i.e. *Arabidopsis thaliana*) has become an important model for studying the genetic control of embryo patterning, growth and development (De Smet *et al*., 2010*a*). The regularity of division planes in arabidopsis embryos is such that mutant phenotypes can easily be discerned based on aberrations in individual cell division planes (Mayer *et al*., 1991; Scheres *et al*., 1994). As will be detailed below, these stereotypical division planes are the result of intricate genetic control, and often generate cells with new, unique gene expression patterns and properties.

The first round of cell division in the embryo is formative in nature (Jürgens and Mayer, 1994). Regulation of cell polarity is important for the asymmetric division of the zygote (Fig. 2B). A zygote divides to generate an embryonic and an extra-embryonic lineage (suspensor). Later, cells in the embryonic lineage divide periclinally to the embryonic surface to generate the protoderm (Fig. 2C). One division round later, a population of inner cells divide periclinally and ground and vascular tissues form (Fig. 2D). Finally, the establishment of the root meristem organizer, the quiescent centre (its precursor is called the 'hypophysis') and the central root cap derives from a typical asymmetric division of the suspensor cell closest to the embryonic lineage (Fig. 2E). Through this series of regulated asymmetric divisions, within a few days a 50-celled embryo is formed that contains tissue precursors, stem cell organizer(s) and presumably also the primordial stem cells.

These processes have long been dissected using forward genetics approaches, and a series of key regulators have thus been identified (reviewed in De Smet *et al*., 2010*a*). However, the number of critical regulators identified through genetics is limited, and it seems that weak mutant phenotypes, feedback and likely feedforward regulation as well as redundancy obstruct the facile identification of new regulatory components. In the following, we will first discuss what 'systems' approaches have been taken in the embryo, and will next exemplify how such strategies have helped our understanding of aspects of regulation in three different formative division events.

Systems approaches in early arabidopsis embryogenesis

Cell type-specific transcript profiling has become an important tool, in particular in post-embryonic root biology. Most studies published to date have used fluorescence-activated cell sorting to isolate cells that are genetically marked by a green fluorescent protein (GFP)-expressing transgene (Birnbaum *et al*., 2003; Brady *et al*., 2007). A critical premise is that the cells to be isolated are accessible or can be easily disassociated. Neither of these is true for embryos, which are encapsulated in the seed coat and fruit. Hence, enzymatic or mechanical disruption is extremely challenging. Several studies have used microdissection to isolate entire embryos of defined stages to determine their transcriptome, and these have helped to provide a global view of the complexity of and changes in transcription in time (Autran *et al*., 2011; Xiang *et al*., 2011; Nodine and Bartel, 2012). However, the absence of tissue specificity is a major limitation, especially for genes expressed in only a few cells or at low levels. To circumvent these issues, several groups have used laser capture microdissection of fixed and embedded fruits to isolate embryo domains for transcript profiling (Casson *et al*., 2005; Spencer *et al*., 2007; Le *et al*., 2010; Belmonte *et al*., 2013). As the amount of RNA and its quality is limited in such an experiment, and because the spatial resolution is not limited to single cell types, the data obtained are useful but do not allow characterization of the molecular transitions associated with cell fate determination. Other methods, such as the two-component nuclear labelling strategy INTACT (Deal and Henikoff, 2010), have the potential to overcome such issues (Palovaara *et al*., 2013). However, at present it is challenging to generate regulatory networks underlying early embryo development in the absence of high-quality cell type-specific transcript data.

The first cell division

After fertilization, the zygote divides to generate a small upper cell and a large lower cell (Fig. 2B). While the upper (apical) cell is the source of most cells in the embryo, the lower (basal) cell forms the filamentous suspensor that positions the embryo within the seed and donates its uppermost derivative to the embryo during root initiation (Hamann *et al*., 1999). Hence, this division creates an embryonic lineage and an extra-embryonic lineage that differ in their cellular division patterns, but also in the expression of several genes (Haecker *et al*., 2004) as well as in their competence to respond to the hormone

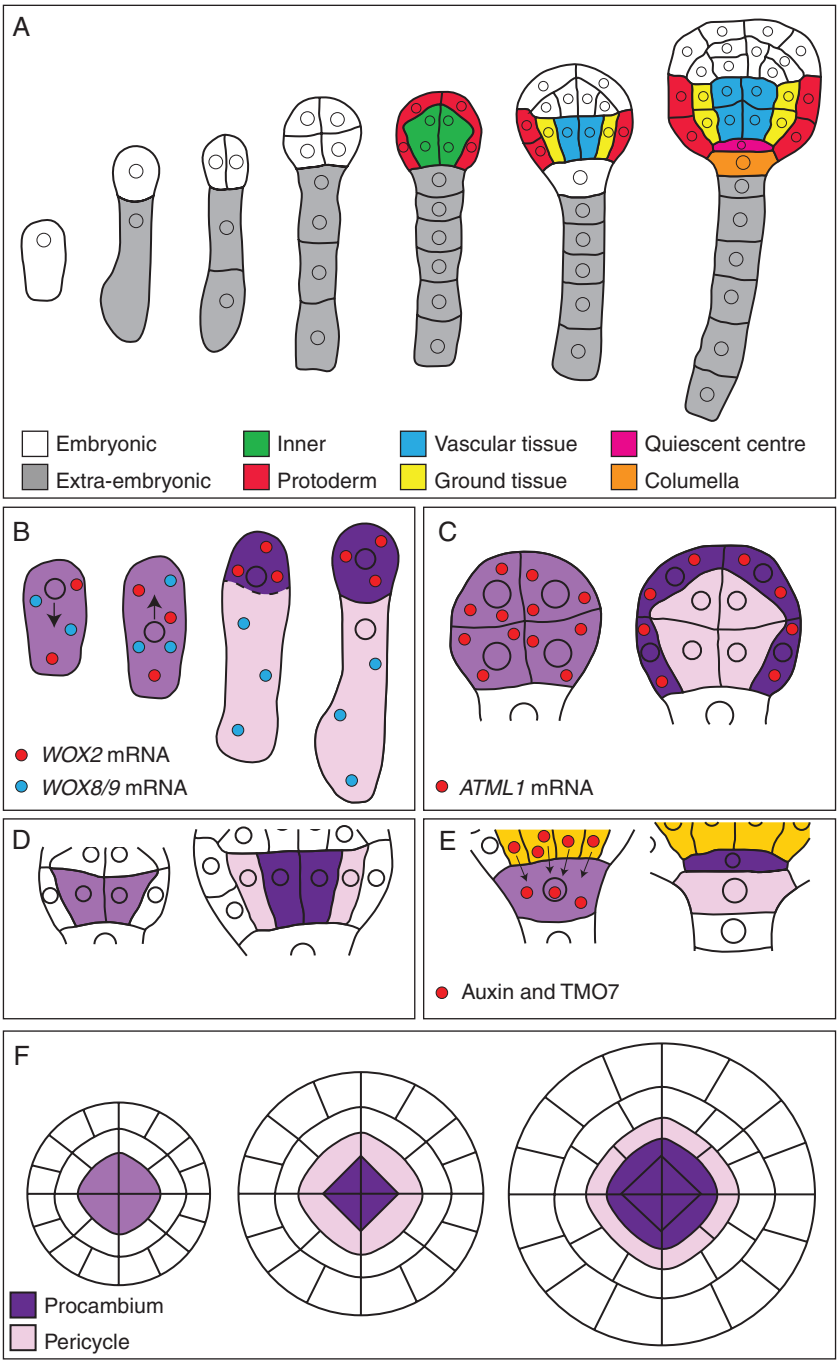

FIG. 2. Asymmetric and formative divisions pattern the arabidopsis embryo. (A) Stages of arabidopsis embryogenesis with the zygote at the left and the transition stage at the right. Cell division patterns are depicted and nuclei are represented as circles. Cell types are coloured as indicated in the legend. (B) Division of the zygote. The egg cell nucleus moves to the centre of the cell shortly after fertilization. Subsequently, the zygote repolarizes and divides along a plane close to the position of the nucleus. This division separates embryonic and extra-embryonic fates. *WOX2* (red) and *WOX8/9* (blue) mRNAs are indicated as spheres. It is not clear whether transcripts segregate or arise *de novo* after zygote division. (C) At the eight-cell stage, all cells undergo a typical asymmetric division that separates protodermal and inner cell identity. Red spheres mark *ATML1* mRNA. (D) Inner cells at the 16-cell stage divide asymmetrically to give rise to vascular (inner) and ground (outer) tissue cells. (E) The upper extra-embryonic (suspensor) cell (also termed hypophysis) divides asymmetrically and generates the precursor to the quiescent centre and the columella root cap initial. Both auxin and TMO7 protein (marked as red spheres) are transported from pro-embryo cells to the hypophysis. (F) Series of cross-sections through the lower hemisphere of 32-cell to late globular stage embryos showing the formative divisions in the vascular tissue that give rise to the establishment of the tissue, as well as the separation of a pericycle layer.

auxin (Rademacher *et al.*, 2011, 2012). Despite its central importance for plant development, the molecular underpinnings of this first, evidently asymmetric, division are largely unknown. Surprisingly few mutations have been found that affect this division (Zhang and Laux, 2011). The egg cell is polarized prior to fertilization; its nucleus is positioned in the upper part and a large

vacuole occupies most of the remaining cell volume. It has long been thought that the asymmetric division follows from this polarization, but recent findings have challenged this simple model. The WRKY2 transcription factor is required for asymmetric zygote division, and acts after fertilization (Ueda *et al*., 2011). By carefully analysing zygote polarity and division in wild-type and *wrky2* mutants, Ueda *et al*. discovered that in wild-type the zygote depolarizes shortly after fertilization (Fig. 2B). This is followed by immediate repolarization. The *WRKY2* gene appears to act in this second step (Ueda *et al*., 2011). Thus, egg cell polarity is not simply carried over to the zygote, but instead the first asymmetric division involves an active repolarization mechanism. Which cues direct repolarization is an unanswered question, but this may involve both intrinsic and extrinsic cues.

The zygote is positioned in a highly polar environment with a cell attachment to the maternal cells on the lower side and the sperm entry site on its upper surface, which could provide extrinsic asymmetry. Segregation of lineage-specific transcripts is in part involved in specification of asymmetric cell identity. WRKY2 is a direct upstream regulator of the *WOX8* and *WOX9* genes (Ueda *et al*., 2011). *WOX8* and *WOX9* encode homeodomain transcription factors that are required for proper specification of suspensor cell identity (Breuninger *et al*., 2008). *WOX8* and *WOX9* mRNAs are co-expressed with the homologous *WOX2* mRNA in the zygote. Upon division, these transcripts are separated into apical and basal cells of the embryo. *WOX2* transcript is limited to the apical cell while *WOX8* and *WOX9* are found in the basal cell (Haecker *et al*., 2004). It remains to be shown that this is based on segregation, not *de novo* transcription, but the reverse-genetic identification of the *WOX* genes and their WRKY2 regulator now allows a systematic dissection of the regulatory network driving asymmetric zygote division. Such dissection will be greatly helped by a better definition of the apical and basal cell transcriptomes, such as has been described in tobacco (Ma *et al*., 2011).

Formative vascular divisions in the embryo

Early during embryogenesis, precursor cells are established for the major tissues. Their numbers are limited and formative divisions are required to increase the population of distinct tissue initials. In the vascular tissue, for example, four precursors are initially specified, yet the final vascular cylinder in the embryonic root encompasses up to 40 cell files (Scheres *et al*., 1994; De Rybel *et al*., 2013), and contains at least four cell types: pericycle, xylem, phloem and procambium (Fig. 2F). A central question is how the formative divisions that generate these cell files are controlled to attain the appropriate and species-specific cell file number. Given the axiality of the embryo, formative divisions in this tissue are by definition periclinal (perpendicular to the surface and axis), while proliferative divisions are anticlinal.

Root development in the embryo strictly depends on the MONOPTEROS/AUXIN RESPONSE FACTOR5 (MP/ARF5) transcription factor (Hardtke and Berleth, 1998). Among the defects in the *mp* mutant is an inability to undergo periclinal vascular divisions (De Rybel *et al*., 2013). A set of likely direct MP targets was identified in a transcript profiling approach (Schlereth *et al*., 2010) and recent characterization of one of these, *TARGET OF MONOPTEROS5* (*TMO5*), revealed a mechanism for local control of periclinal, formative division. *TMO5* encodes a basic helix–loop–helix (bHLH) transcription factor and is activated in all procambial cells by MP (Schlereth *et al*., 2010). A double mutant with its closest homologue TMO5-LIKE1 (T5L1) causes a 2-fold decrease in vascular cell file number through impaired periclinal division (De Rybel *et al*., 2013). There is a striking similarity to the phenotype of the *lonesome highway* (*lhw*) mutant, also affected in a bHLH transcription factor (Ohashi-Ito and Bergmann, 2007). A proteomics screen for TMO5 protein complex components identified LHW as its dimeric partner (De Rybel *et al*., 2013), and genetic analysis suggests that dimerization is critical for function (De Rybel *et al*., 2013). Both TMO5 and LHW have multiple close homologues that also heterodimerize, suggesting highly redundant functions. Indeed, higher-order mutations lead to complete loss of vascular periclinal cell divisions and loss of the tissue. The overlap of TMO5 and LHW protein accumulation marks a small domain in embryo and root vascular tissue, and overlaps well with the domains in which periclinal divisions occur. Strikingly, misexpressing both TMO5 and LHW together is able to trigger periclinal divisions in all other cell types of the root (De Rybel *et al*., 2013). This suggests that the overlapping patterns of TMO5 and LHW protein accumulation define where asymmetric divisions are initiated in the plant. Given that *TMO5* and *LHW* encode transcription factors, further genetic control will be involved in determining cell division orientation. Nonetheless, this is a clear example of how systematic target gene identification and proteomics have identified a protein complex that integrates developmental cues to locally trigger a formative division.

Asymmetric hypophysis division

The division that gives rise to the quiescent centre (QC) and the columella initial cell is highly asymmetric (Fig. 2E). The plane of division of the dome-shaped hypophysis cell is asymmetric, such that the upper cell is much smaller than the lower cell. The plane of this division is crucial for root initiation, and auxin response is required for its correct execution. The auxin response marker *DR5-GFP* is expressed in the hypophysis prior to its division (Friml *et al*., 2003) and mutations that interfere with auxin response inhibit the asymmetric division (Weijers *et al*., 2006; Rademacher *et al*., 2012). The machinery responsible for this division asymmetry has not yet been addressed, but recent work has led to the identification of signals that promote this division or the specification of cell fates after division. The division requires MP activity. However, MP is expressed and acts in the adjacent embryonic cells, which suggested non-cell-autonomous action and potentially extrinsic or niche-controlled asymmetric division (Weijers *et al*., 2006). The intercellular signalling between the embryo proper and the hypophysis involves directional transport of auxin. The auxin transporter PIN1 localizes to the lower membrane of the embryonic cells and PIN1 expression is lost in the *mp* mutant (Weijers *et al*., 2006).

Auxin response in the suspensor is not limited to the uppermost cell, but extends to one or two cells subtending it (Friml *et al*., 2003; Weijers *et al*., 2006). Hence, auxin response alone is unlikely to be sufficient for the establishment of this asymmetric cell division. In a microarray-based screen for MP targets, the

TMO7 bHLH transcriptional regulator was identified (Schlereth *et al*., 2010). *TMO7* transcription is directly controlled by MP in the embryonic cells, and RNAi suppression of *TMO7* causes *mp*-like rootless defects. Localization of the TMO7–GFP protein showed that it moves to the adjacent hypophysis cell. In support of a role for TMO7 movement in hypophysis establishment, the *mp* mutant defect could be partially suppressed by providing TMO7 protein in suspensor cells (Schlereth *et al*., 2010). Thus, the specification and asymmetric division of the hypophysis root precursor relies on two directionally transported mobile signals: auxin and TMO7 protein. Again, a genome-wide transcript profiling approach has allowed the identification of novel extrinsic signals in asymmetric cell division.

GROWING GROUND TISSUE: THE CORTEX ENDODERMIS INITIAL CELLS

There is increasing evidence that the maintenance of stem cells in plant post-embryonic tissue is controlled by signals from the local microenvironment, commonly known as the stem cell niche (Scheres, 2007). The stem cell niche in the arabidopsis root consists of the vascular initials, epidermis/lateral root cap initials, columella initials, cortex/endodermis initials (CEI), and the QC cells. The QC cells function to regulate and maintain the surrounding stem cells in their undifferentiated state (van den Berg *et al*., 1995). The initial cells continually undergo stereotypic asymmetric divisions to produce daughter cells that are sequentially displaced from the stem cell niche and start to differentiate. The overall result of this process is organized cell layers where entire cell lineages, from stem cells to differentiated progeny, are constrained spatially and longitudinally within files. In some cases, asymmetric divisions of distinct initial cells are coordinated temporally. For instance, the division of the epidermis/lateral root cap initial is coordinated with that of the columella initials to produce an organized root tip that protects the stem cell niche (Wenzel and Rost, 2001; Baum *et al*., 2002). Of all the asymmetric divisions that occur in the root stem cell niche, those of the CEI are best described at the molecular level using a combination of genomics and modelling approaches. SHORT-ROOT (SHR) and SCARECROW (SCR) regulate the asymmetric divisions of CEI cells that give rise to the endodermal and cortical layers, collectively called the ground tissue. CEI cells undergo two asymmetric divisions, one anticlinal division to reproduce itself and a CEI daughter cell (CEID), and one periclinal division of the newly created CEID that leads to the formation of the ground tissue.

Early genetic screens and molecular analyses revealed the location and function of the mobile GRAS family transcription factor SHR and its downstream target, SCR (DiLaurenzio *et al*., 1996). The SHR protein is initially produced in the vasculature and moves to the QC, CEI and endodermis, where it upregulates expression of *SCR* (Helariutta *et al*., 2000; Nakajima *et al*., 2001; Gallagher *et al*., 2004; Levesque *et al*., 2006; Cui *et al*., 2007; Gallagher and Benfey, 2009). Mutations in either SHR or SCR result in the formation of a single ground tissue layer. While *scr* mutants express both endodermis and cortex specific markers, *shr* mutants express only cortex-specific markers. Both SHR and SCR are involved in the regulation of formative divisions, but only SHR is required for endodermal specification. However, the role of SHR in specification of the asymmetric identity of CEID cells is less clear (Benfey *et al*., 1993; DiLaurenzio *et al*., 1996). SCR limits the number of endodermal cell layers through the regulation of SHR movement. *SCR* RNAi lines, expressing reduced levels of SCR, show increased movement of SHR and the formation of a third layer within the ground tissue. Thus, SCR blocks SHR movement and any subsequent formative divisions (Cui *et al*., 2007). Accordingly, SCR directly interacts with SHR, which leads to the sequestration of SHR into the nucleus of endodermal cells (Cui *et al*., 2007). Additionally, it was shown that SCR binds its own promoter and thus autoregulates (Sabatini *et al*., 2003; Heidstra *et al*., 2004; Cui *et al*., 2007). Recently, another protein, SHORT-ROOT INTERACTING EMBRYONIC LETHAL (SIEL), has been shown to interact with SHR and to promote its movement (Koizumi *et al*., 2011). The C2H2 zinc finger transcription factors JACKDAW (JKD) and MAGPIE (MGP) also interact with SHR and SCR and may affect SHR movement. Indeed, JKD alters SCR expression in the ground tissue (Heidstra *et al*., 2004). Mutations in *JKD* result in a third layer within the ground tissue as a consequence of periclinal divisions in the cortical cells (Welch *et al*., 2007). A reduction in *MGP* activity can partially rescue the *jkd* phenotype, thus suggesting that MGP opposes the role of JKD in the SHR/SCR network (Welch *et al*., 2007). Since MGP is negatively regulated by RETINOBLASTOMA-RELATED (RBR), a protein shown to promote cellular differentiation, it was thought that RBR might interact with SHR and SCR (Welch *et al*., 2007). Bimolecular fluorescence complementation assays showed that RBR binds to SCR through a specific amino acid motif, the LxCxE motif (Cruz-Ramirez *et al*., 2012). RBR binding to SCR through its LxCxE motif spatially restricts formative divisions while disruption of this binding leads to the formation of an additional cell layer in the ground tissue (Cruz-Ramirez *et al*., 2012, 2013). These results suggest that RBR interaction with SCR counteracts the ability of SCR to induce formative divisions (Cruz-Ramirez *et al*., 2012) (Fig. 3).

Omics and modelling approaches have been elegantly used to characterize the main players that regulate asymmetric cell divisions in ground tissue. In order to identify genes responsible for cell division in the CEIs and their daughter cells, *SHR* and *SCR* expression was induced in an *SHR* mutant background and ground tissue cells were isolated at several time points after induction using fluorescence-activated cell sorting and microarray analysis. The cell cycle regulator CYCLIND6;1 (CYCD6;1) was identified and confirmed as a direct transcriptional target of SHR by chromatin immunoprecipitation (Sozzani *et al*., 2010). CYCD6;1 was shown to inactivate RBR by phosphorylation, therefore indirectly influencing SCR activity (Sozzani *et al*., 2010; Cruz-Ramirez *et al*., 2012). Additional computational modelling approaches have been used in an iterative fashion to aid our understanding of the dynamic interactions of SHR, SCR, RBR and CYCD6;1. Specifically, the use of mathematical models has led to the non-intuitive prediction that in the stem cell niche high levels of auxin would bias the circuit in favour of high SHR–SCR activity and a subsequent formative cell division. When tested, these predictions suggested the presence of bistability due to a nested positive feedback loop (SCR positive feedback loop and CYCD6 repression of the RBR repressor) (Cruz-Ramirez *et al*., 2012; Sozzani and Iyer-Pascuzzi, 2014). The positive influence of auxin focuses CYCD6;1 expression

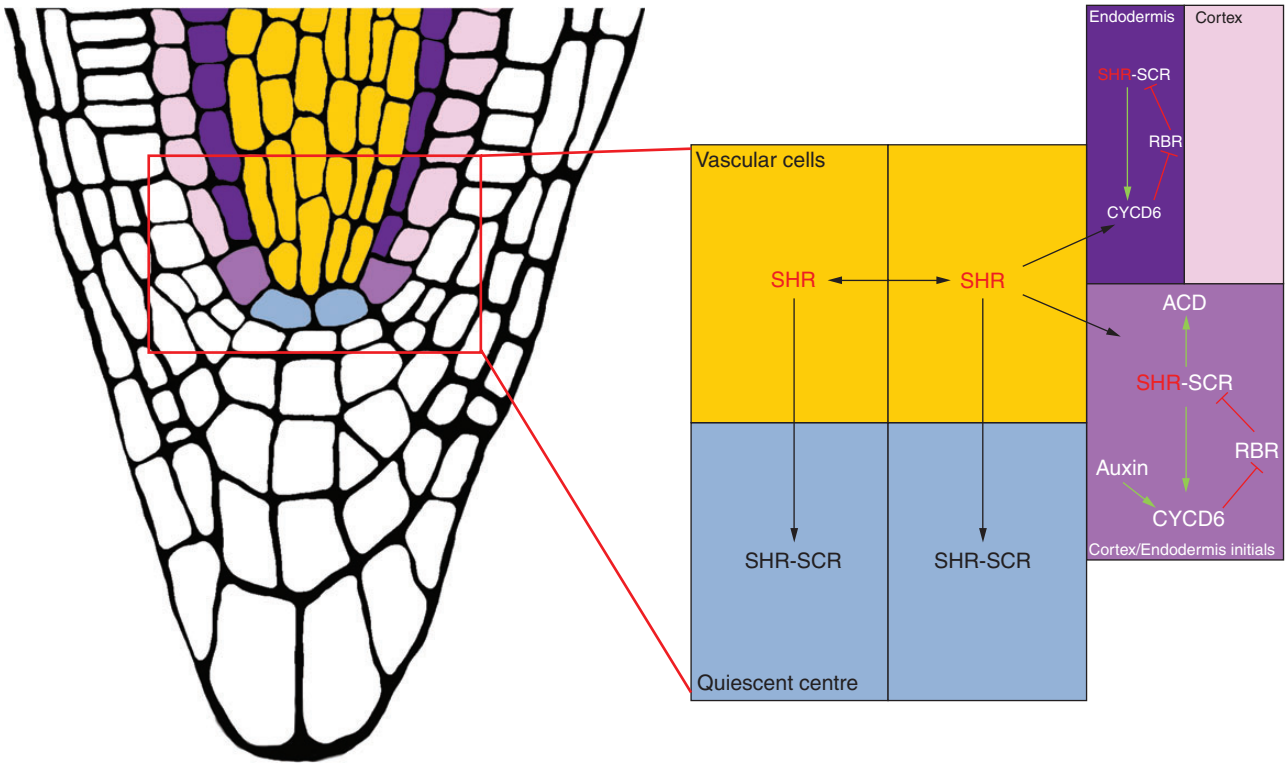

FIG. 3. The cortex/endodermis initial asymmetric division. Schematic representation of the SHR/SCR/CYCD6/RBR network, which regulates the cortex/endodermis initial (CEI) asymmetric cell division that give rises to both the cortical and the endodermal layer. The mobile transcription factor SHR moves from the vasculature to the adjacent cells (quiescent centre, CEI and endodermis), where it activates transcription of SCR. SHR and SCR interact and subsequently activate CYCD6 expression, which negatively regulates RBR by post-translational modification, namely phosphorylation. A high auxin gradient biases CYCD6 expression in the CEIs and limits the asymmetric cell division taking place only in the CEI/D. Black arrows represent protein movement, red represents repression and green represents activation.

in the CEI/D and the bistability due to the nested feedback loop ensures that the formative cell division will only take place in the CEI/D. Together, this example of multiple approaches and the acquisition of cell type and temporal stage-specific data beautifully illustrate the power of these techniques to identify more players that regulate formative asymmetric divisions.

Despite much advancement in our understanding of how specific networks work to control different stem cell populations in their cellular context, a comprehensive model of the extrinsic nature of the QC on regulating asymmetric divisions of initial cells as a whole is still missing. Describing how regulatory signals propagate across cells and finding the link between networks sustaining different stem cells will be important contributions to our understanding of stem cell niche maintenance.

ASYMMETRIC CELL DIVISIONS DURING LATERAL ROOT INITIATION

Lateral roots are key contributors to root system architecture that arise post-embryonically from the primary root as a result of several asymmetric cell divisions (Peret *et al.*, 2009; De Smet, 2012; Smith and De Smet, 2012). In arabidopsis, a limited, prespecified set of pericycle cells adjacent to a xylem pole will undergo asymmetric, anticlinal cell divisions (referred to as 'lateral root initiation') to form unequally sized daughter cells (De Smet *et al.*, 2008). This is followed by a 90° shift in the axis of division upon which the small daughter cells divide periclinally in an outward manner to form a core of cells with different identities (Malamy and Benfey, 1997; Dubrovsky *et al.*, 2001) (Fig. 4). Through a series of cell divisions and differentiation steps, a lateral root primordium is formed that eventually emerges through overlying tissues of the primary root. Thus, a new stem cell niche, which in turn controls the further growth of the lateral root, is then generated (Malamy and Benfey, 1997; Swarup *et al.*, 2008; Peret *et al.*, 2009; De Smet and Beeckman, 2011; Goh *et al.*, 2012; De Smet, 2012; Lavenus *et al.*, 2013; Lucas *et al.*, 2013*a*). Intriguingly, tissues overlaying a lateral root primordium influence the shape of the primordium (Lucas *et al.*, 2013*a*). Here we highlight what pre-initiation events and anticlinal and periclinal asymmetric cell divisions are taking place during early stages of lateral root development. Several events need to occur sequentially, or need to come together in a small number of pericycle cells, before asymmetric cell division occurs, such as priming, cell cycle progression, founder cell establishment, symmetry breaking, nuclear migration and auxin response.

Before a morphologically distinct asymmetric cell division

Developmental decisions on the distribution of lateral roots take place in the distal zone of the root tip in the basal meristem region (Fig. 4). Studies using a *DR5::GUS* (an auxin-responsive

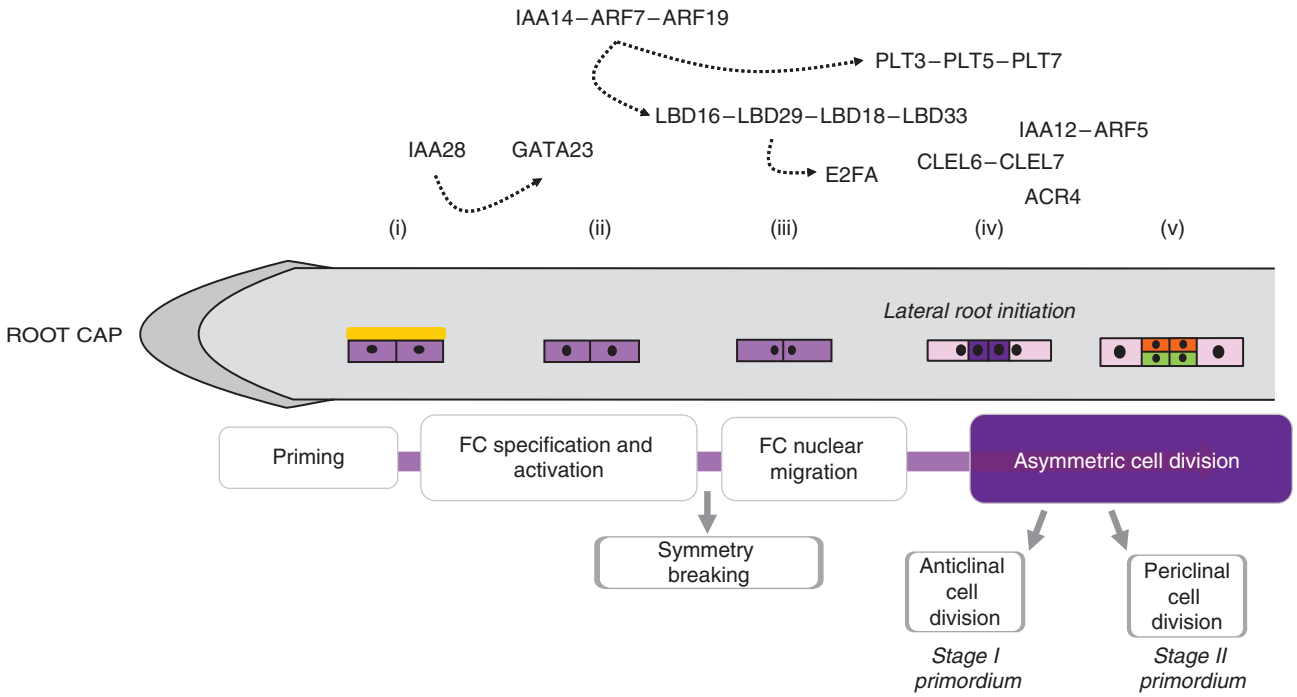

FIG. 4. Schematic representation of the primary root of arabidopsis indicating the developmental progression along the primary root axis (pre-initiation events) leading to asymmetric cell division (ACD). (i) Priming stage in basal meristem region, in which generation of an auxin maximum (yellow) in the protoxylem cells alongside oscillating gene expression in the region leads to formation of prebranch sites in the adjacent xylem pole pericycle cells (XPPs). (ii) These XPP cells have the competence to form lateral roots and pass through a developmental window to become specified founder cells (FCs). (iii) Migration of nuclei towards the common cell wall indicates FCs preparing to undergo asymmetric cell division. (iv) Anticlinal asymmetric cell division of FCs occurring in the differential zone of the root, giving rise to two small daughter cells (deep purple) and two larger flanking cells (pale purple), also representing the stage I primordium. (v) Following anticlinal division, the axis of division shifts by 90°, with the central core of small daughter cells [represented by deep purple in (iv)] dividing in an outward manner, giving rise to equally sized daughter cells with different identities (orange and green), also representing stage II primordium. A selection of key players regulating these processes is indicated for the distinct steps.

marker) time series (De Smet *et al*., 2007; De Rybel *et al*., 2010) and *in vivo*, real-time visualization of *DR5::LUCIFERASE* expression, together with genome-wide gene expression studies (Moreno-Risueno *et al*., 2010), suggest that periodic auxin response, along with oscillating waves of gene expression, functions as an endogenous clock-like mechanism. The clock output is the formation of prebranch sites that are future sites of lateral root primordia in a regular pattern along the primary root (De Smet *et al*., 2007; Moreno-Risueno *et al*., 2010; Van Norman *et al*., 2013) (Fig. 4). An auxin response maximum occurs in xylem elements adjacent to the xylem pole pericycle (XPP) cells in the root basal meristem. These are the cells that get primed, providing them with the competence to undergo lateral root initiation (De Smet *et al*., 2007; Parizot *et al*., 2008, 2012). Interestingly, the cytokinin response is repressed in the priming and initiation region of the root. Accordingly, ectopic cytokinin overproduction in the basal meristem is inhibitory to lateral root initiation (Bielach *et al*., 2012). To further explore these developmental decisions in the basal meristem, using a marker-based chemical biology approach, a non-auxin like molecule, named naxillin, was identified (De Rybel *et al*., 2012). This chemical promotes auxin response specifically in the basal meristem region, where founder cell identity is established, and can be used as a tool to unravel the molecular networks surrounding priming and founder cell identity establishment.

Under controlled growth conditions, the primary root displays a root-waving pattern. The left–right positioning of the lateral roots along the primary root axis correlates with the root curvature, with the lateral roots forming on the convex sides of the bends in the root (Fortin *et al*., 1989; De Smet *et al*., 2007; Ditengou *et al*., 2008; Laskowski *et al*., 2008; Lucas *et al*., 2008). Bending of the root induces a mechanical strain, which exhibits activation in local competence of XPP cells caused by changes in local auxin distribution and signalling (Ditengou *et al*., 2008; Laskowski *et al*., 2008; Laskowski, 2013). However, expression of the auxin-responsive *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*) suggests that an auxin response maximum may occur in XPP cells before the root bends (Bishopp *et al*., 2011).

GATA23 is the earliest known marker for lateral root development that was identified by meta-analysis of transcriptomic data sets for lateral root initiation (Parizot *et al*., 2010; De Rybel *et al*., 2010). *GATA23* is expressed in XPP cells before the first asymmetric division. The *GATA23* RNAi line shows a decrease in the number of emerged and non-emerged primordia. On the other hand, *GATA23* overexpression caused an increase in the number of founder cells observable by a high occurrence of ectopic primordia (De Rybel *et al*., 2010). An AUXIN/INDOLE-3-ACETIC ACID28 (AUX/IAA28)-dependent auxin signalling mechanism controls *GATA23* expression in the basal meristem, regulating lateral root founder cell specification

prior to lateral root initiation and root waving (Fig. 4) (Rogg *et al*., 2001; Brady *et al*., 2007; De Rybel *et al*., 2010; Yadav *et al*., 2010).

Subsequent to priming, XPP cells pass through a developmental window for lateral root initiation in which, at minimum auxin concentration, the XPP cells have a high probability of becoming specified founder cells (Fig. 4) (Dubrovsky *et al*., 2008, 2011). The endodermis assists in the transition from the founder cell state to the lateral root initiation phase via an auxin reflux pathway between endodermal cells and the adjacent founder cells. This is achieved through laterally localized *PIN3* expression in overlaying endodermal cells reinforcing auxin movement to founder cells. The *pin3* mutant exhibits a dramatic delay in the onset of lateral root initiation following founder cell specification (Marhavy *et al*., 2013). PIN7 also regulates founder cell specification, with a mutation in *pin7* affecting the number of primordia (Benková *et al*., 2003; Marhavy *et al*., 2013). Taken together, these findings show that auxin accumulation through AUXIN RESISTANT1 (AUX1, an auxin influx carrier)–PIN based transporter activity and their positive feedback and cross-regulation control lateral root initiation both intrinsically (establishment of an auxin maximum within the founder cells) and extrinsically (auxin flux from the endodermis) (Laskowski *et al*., 2008). When a local auxin concentration maximum is reached, the cells proceed to lateral root initiation (Dubrovsky *et al*., 2008; Benková *et al*., 2009). Cytokinins also affect this process by repressing many PIN proteins, thereby preventing the proper auxin gradient establishment that is required for respecification of lateral root founder cells (Li *et al*., 2006; Laplaze *et al*., 2007; Chang *et al*., 2013). Recent studies suggest that cytokinin may aid as a positional signal for the formation of new lateral root primordia (Chang *et al*., 2013).

Transition from founder cell to lateral root initiation

Following the increased auxin response in lateral root founder cell pairs, several AUX/IAA–ARF factors interact together. This leads to the migration of nuclei towards the common cell wall, a morphological feature of lateral root founder cells preparing for asymmetric cell division (Fig. 4) (De Smet *et al*., 2007; De Rybel *et al*., 2010). The SOLITARY ROOT (SLR)/IAA14–ARF7–ARF19 module regulates this synchronous migration (De Smet *et al*., 2007; Dubrovsky *et al*., 2008; De Rybel *et al*., 2010; Goh *et al*., 2012). The *slr-1* and *arf7 arf19* mutants, when treated with excess auxin, show abnormal nuclear migration and asymmetric cell divisions (De Rybel *et al*., 2010). Lateral root initiation closely follows the nuclear migration in the differentiation zone (Dubrovsky *et al*., 2011). In parallel, pericycle cells need to swell radially before undergoing asymmetric cell divisions (Vermeer *et al*., 2014). It is essential that the overlying endodermis accommodates this, as blocking the auxin response in the endodermis leads to the absence of asymmetric pericycle cell divisions (Vermeer *et al*., 2014).

Control of cell cycle activity is crucial for lateral root founder cells to undergo asymmetric cell divisions (Beeckman *et al*., 2001; Himanen *et al*., 2002; DiDonato *et al*., 2004; Vanneste *et al*., 2005; Jurado *et al*., 2010; De Smet *et al*., 2010*b*; Sanz *et al*., 2011). It is key that the cells remain in a mitotically competent state prior to an asymmetric cell division, which is suggested to be maintained by the *ABERRANT LATERAL ROOT FORMATION 4* (*ALF4*)-encoded nuclear protein (with the *alf4* mutant displaying no lateral root initiation) (Beeckman *et al*., 2001; DiDonato *et al*., 2004; Dubrovsky *et al*., 2008). The transition of XPP cells from G1 to S and the ensuing cell cycle progression are stimulated by auxin. These 'primed' cells reactivate the cell cycle only when they reach the lateral root initiation zone (Casimiro *et al*., 2003), emphasizing the fact that activating cell cycle-related genes alone is not sufficient to initiate a new lateral root (Vanneste *et al*., 2005). Blocking either early auxin response (in a gain-of-function *slr* mutant) or transport [using the transport inhibitor *N*-1-naphthylphthalamic acid (NPA)] results in the absence of lateral root initiation (Casimiro *et al*., 2001; Himanen *et al*., 2002; Vanneste *et al*., 2005). Gain-of-function mutations in SLR/IAA14 (Fukaki *et al*., 2002, 2005; Vanneste *et al*., 2005) and IAA28 (Rogg *et al*., 2001; Dubrovsky *et al*., 2009; De Rybel *et al*., 2010) negatively regulate lateral root formation through the inactivation of ARF7 and ARF19, which are necessary for activation of lateral root initiation genes. The *slr* mutants are agravitropic and show a complete lack of lateral roots (Fukaki *et al*., 2002); additionally, *arf7 arf19* double mutants exhibit fewer lateral roots owing to inhibition of the auxin-induced pericycle cell division that is required for lateral root initiation (Wilmoth *et al*., 2005; Okushima *et al*., 2007; Dubrovsky *et al*., 2009). ARF7 and ARF19 together activate LATERAL ORGAN BOUNDARIES-DOMAIN16/ASYMMETRIC LEAVES2-LIKE18 (LBD16/ASL18) and LBD29/ASL16 (Okushima *et al*., 2007), with *LBD16* being expressed specifically in the lateral root founder cells before the first asymmetric cell division. LBD16/ASL18, along with other related LBDs, regulate the establishment of asymmetry of the founder cell (Goh *et al*., 2012). The E2Fa transcription factor promotes asymmetric cell divisions during lateral root initiation (De Smet *et al*., 2010*b*; Berckmans *et al*., 2011). *E2Fa* expression is regulated by the LBD18–LBD33 dimer, which is in turn regulated by the auxin signalling pathway (Berckmans *et al*., 2011). The auxin-mediated G1-to-S transition is inhibited by the INTERACTOR OF CYCLIN-DEPENDENT KINASE (CDK)/KINASE-INHIBITORY PROTEIN (KIP)-RELATED PROTEIN (ICK/KRP) family of proteins, with the *krp2* mutants showing increased lateral root density. Activation of the cell cycle occurs by the formation of a complex with ICK2/KRP2 and CDKA;1–CYCD2;1, thus inhibiting the activity of ICK2/KRP2 and increasing the activity of CDKA;1–CYCD2;1 and subsequently increasing lateral root density (Himanen *et al*., 2002; Ren *et al*., 2008; Sanz *et al*., 2011). Other D-type cyclin (CYCD) subunit genes (*CYCD4;1* and *CYCD3;1*) are also known to be involved in lateral root initiation (Himanen *et al*., 2002). In addition, A2-type cyclins (CYCA2 s) are involved in early G2-to-M transition of the cell cycle during lateral root initiation. Accordingly, a triple *cyca2;234* mutant show a delay in the expression of mitotic regulators, while the auxin signalling and G1-to-S regulatory genes remain unaffected (Vanneste *et al*., 2011). S-PHASE KINASE-ASSOCIATED PROTEIN 2A (SKP2A), a cell cycle F-box protein, positively regulates lateral root initiation. Auxin binds directly to SKP2A and regulates the proteolysis of cell cycle-repressing transcription factors in a TRANSPORT INHIBITOR RESPONSE 1 (TIR1)–AUXIN SIGNALING F BOX PROTEIN (AFB) auxin receptor-independent pathway. Overexpression of SKP2A in the *tir1* mutant background induces lateral root initiation and *skp2a*

mutants show an auxin-resistant root growth phenotype (Jurado *et al*., 2008, 2010). A close homologue, SKP2B, is contrastingly involved in negative regulation of cell division in founder cells. *skp2b* mutants display higher numbers of stage I and II primordia, suggesting involvement of *skp2b* in the first asymmetric cell division (Manzano *et al*., 2012). Finally, in addition to auxin, cytokinins act directly on lateral root founder cells, blocking the G2–M cell cycle transition (Li *et al*., 2006). The positioning of lateral root initiation sites is also controlled by PLETHORA3 (PLT3), PLT5 and PLT7, transcription factors that are expressed in the founder cells. Their activity (downstream of ARF7 and ARF19) restricts the region of lateral root initiation to a single focus, ensuring that clusters of adjacent or opposite lateral roots are not formed (Hofhuis *et al*., 2013).

Anticlinal asymmetric divisions of pericycle cells

The pair of founder cells undergo the first formative anticlinal asymmetric cell divisions, giving rise to two small daughter cells and two larger flanking cells – a stage I primordium (Fig. 4) (De Smet *et al*., 2008). This is an exception to the generality in roots that asymmetric formative divisions are largely periclinal. This pattern of asymmetric cell division occurs simultaneously in two or three pairs of adjacent pericycle cell files (Kurup *et al*., 2005). The small daughter cells are characterized by the differential expression of several genes. For example, *ARABIDOPSIS CRINKLY4* (*ACR4*), encoding a receptor-like kinase, was identified through an integrated systems biology approach involving transcript profiling of auxin-activated XPP cells in a highly synchronized time course subjected to fluorescence-activated cell sorting (De Smet *et al*., 2008). This led to a list of differentially expressed genes potentially involved in lateral root initiation with high spatiotemporal resolution and associated with asymmetric cell division. *ACR4* was one of the main candidates identified (De Smet *et al*., 2008). *ACR4* promotes a first formative asymmetric, anticlinal cell division in two neighbouring pericycle cells, resulting in a small and large flanking cell (De Smet *et al*., 2008).

Following the first asymmetric division, the small daughter cells exhibit an auxin maximum (Benková *et al*., 2003), which occurs around the time when BDL/IAA12-MP/ARF5-dependent signalling occurs (De Smet *et al*., 2010*b*). The BDL/IAA12–MP/ARF5-dependent auxin response helps patterning of the lateral root downstream of SLR/IAA14–ARF7–ARF19. Accordingly, the hemizygous gain-of-function *bdl* mutants (*ProBDL:bdl*^{hem}) and weak loss-of-function *mp*^{S319} mutants show abnormalities in pericycle divisions and irregularly positioned lateral roots (De Smet *et al*., 2010*b*). Interestingly, the presence of ARF7–ARF19-independent lateral root formation is revealed by REDUCED LATERAL ROOT FORMATION, a cytosolic protein with a cytochrome b_5-like haem/steroid binding domain (Ikeyama *et al*., 2010). This is evident by the *rlf-1* mutation having an inhibitory effect on anticlinal cell division of the founder cells, thereby affecting lateral root initiation. However, auxin-inducible expression of *LBD16/ASL18* and *LBD29/ASL16* genes remains unaffected in the *rlf-1* mutant. Some *ROOT GROWTH FACTOR* (*RGF*)*/GOLVEN (GLV)/CLE-LIKE* (*CLEL*) genes, encoding small signalling peptides, are expressed in stage I primordia (Fernandez *et al*., 2013*a*, *b*). *CLEL6* or *CLEL7* affect the regular pattern of asymmetric cell division leading to lateral root primordium formation, suggesting the possibility of their role in later asymmetric divisions (Meng *et al*., 2012).

Periclinal asymmetric division of pericycle cells

Subsequent to the anticlinal divisions, the cell division plane shifts by 90° for the next asymmetric cell division. Here, cells divide in an outward (periclinal) manner forming a second layer (stage II primordium) (Fig. 4) (Malamy and Benfey, 1997; De Smet *et al*., 2008). Strict control of cell division orientation is required for *de novo* organogenesis and shape formation. Several proteins are implicated in this process (De Smet and Beeckman, 2011; Rasmussen *et al*., 2011). Recently α-Aurora kinases (AUR1 and AUR2), which were also identified in the systems approach mentioned above for ACR4 (De Smet *et al*., 2008), have been found to influence the mechanism by which the switch in the plane of division occurs during asymmetric cell division in lateral roots. The *aur1-2 aur2-2* double mutants exhibit randomly oriented cell divisions instead of distinct cell layers during primordium development, and a change in growth dynamics that affects lateral root development. However, the dome shape of the lateral root primordia that develop and that display irregular cell division patterns remained unaffected (Van Damme *et al*., 2011; Lucas *et al*., 2013*a*). Evidence of difference in cell identity between the distinct cell layers after the periclinal division is provided by the End199 GUS marker, and this reflects activation of endodermis identity in addition to the distinction between the inner and outer layer of cells in the stage II primordium. This is also one of the earliest observed instances of differential expression between the cell layers (Malamy and Benfey, 1997).

VASCULAR PROCAMBIUM AND CAMBIUM ASYMMETRIC CELL DIVISIONS

Vascular procambium and cambium cells are a lateral or dispersed stem cell niche found in vascular plants (Esau, 1965). Procambial cells are responsible for radial primary growth producing primary vascular tissues, while cambial cells are responsible for secondary growth leading to radial thickening (Esau, 1965). Procambium can be found in stems, leaves, hypocotyl and roots. In all cases these cell populations undergo asymmetric cell divisions to produce additional layers of xylem or phloem. These cells can be considered as asymmetric amplifying cells such that they produce one daughter cell, which will continue to have procambial or cambial cell identity and another daughter cell with either phloem cell identity or xylem cell identity. The patterning and position of cells within vascular bundles differs depending on organ and developmental stage. Primary stems contain multiple vascular bundles, arranged circumferentially (Esau, 1965) (Fig. 5A). Procambium is located across the diameter of each bundle (parallel to the stem epidermis) as intervening tissue between phloem and xylem. After cells originating from primary meristems have elongated and matured, primary growth stops. At the start of secondary growth in the stem, fascicular cambium is induced from procambium and interfascicular cambium is induced from interfascicular parenchyma located between the vascular bundles, leading to secondary stem where the patterning and position of the cambium changes. Here, vascular cambium is a concentric cylinder of tissue,

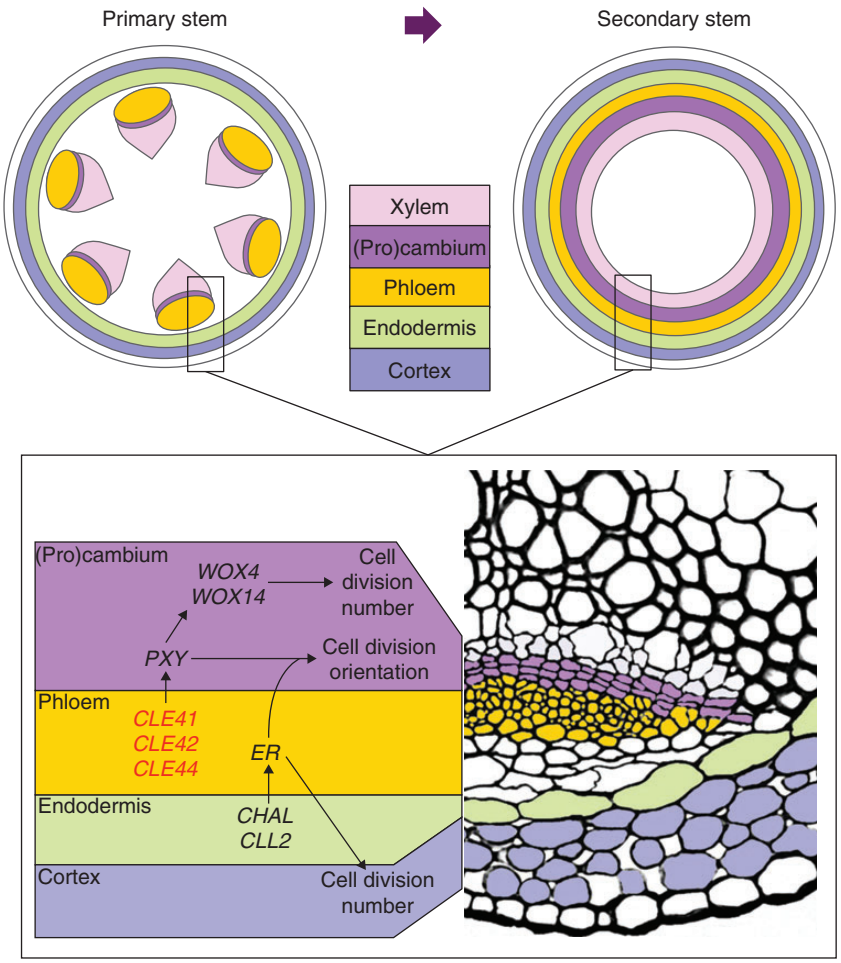

FIG. 5. Procambium and cambium asymmetric divisions. (A) Diagram of a cross-section of primary and secondary stems. Primary stems have vascular bundles where asymmetric divisions of procambium (also called fascicular cambium) produce cells for procambium, phloem and xylem. When the stem transitions from primary to secondary growth, interfascicular cambium is induced, which allows cambium, phloem and xylem to form continuous rings around the stem. (B) Schematic showing the known interactions in the PXY/TDR signalling pathway across the cell types in the stem.

located between xylem and phloem cells. Together, these three tissues (vascular cambium, xylem and phloem) generally form continuous concentric rings (Esau, 1965) (Fig. 5A). In arabidopsis, secondary growth occurs at the base of the inflorescence stem and in the root in their final growth stages, as well as hypocotyls after bolting (Ragni *et al*., 2011), and in *Populus*, a model organism for cambium development in trees, secondary growth gives rise to the bulk of the woody tissue in the stem. The root procambium and cambium are similarly located between the xylem and phloem cells, and carry out the identical function of dividing asymmetrically to produce more cells for all three cell types (Fig. 5). Here we will concentrate on the current understanding of niche-controlled stem cell identity determination in procambial and cambial symmetric cell divisions, based on work carried out mainly in arabidopsis inflorescence stems, but also in arabidopsis hypocotyls and roots and poplar (*Populus* spp.) stems.

Extrinsic signalling determines the orientation and (pro)cambial cell proliferation . . .

The pathways promoting asymmetric cell division are conserved in many of the angiosperm stem cell niches. Vascular procambium and cambium, just like the organizing centre of the shoot apical meristem (SAM) and root QC, use a short mobile peptide signal, CLAVATA3/ESR-related (CLE), a receptor-like kinase and a WUSCHEL-related homeobox (WOX) transcription factor to promote stem cell identity in a niche- or extrinsically controlled fashion (Laux *et al*., 1996; Mayer *et al*., 1998; Schoof *et al*., 2000; Sarkar *et al*., 2007; De Smet *et al*., 2008; Nardmann and Werr, 2013), as shown in Fig. 5B.

The procambium-mobile CLE peptides were first detected in *Zinnia elegans* mesophyll-to-tracheary element transdifferentiating *in vitro* cultures. A 12-amino acid CLE peptide was identified in the extracellular fraction and named after its function as tracheary element differentiation inhibitory factor (TDIF) (Ito *et al*., 2006). Its close homologues in arabidopsis, CLE41/44 and CLE42, are also capable of repressing tracheary element differentiation (Ito *et al*., 2006) and promoting the proliferative capability of procambium and cambium cells (Whitford *et al*., 2008). Interestingly, these 12-amino acid peptides are synthesized and secreted from phloem, a daughter cell type resulting from an asymmetric division of a procambial or cambial cell (Hirakawa *et al*., 2008).

The receptor interacting with these CLE peptides is synthesized in procambial cells adjacent to phloem cells. The procambium-expressed receptor-like kinase PHLOEM INTERCALATED WITH XYLEM (PXY) was first identified as a regulator of the orientation of the procambial division in arabidopsis inflorescence stem (Fisher and Turner, 2007), and again later as the procambial-expressed TDIF RECEPTOR (TDR) by homology with leucine-rich repeat receptor-like kinases (LRR-RLKs) that recognizes CLE peptides (Hirakawa *et al*., 2008). The interaction of PXY/TDR with phloem-derived CLE41 regulates the procambial and cambial division orientation in inflorescence stems and hypocotyls (Etchells and Turner, 2010). However, PXY is not the sole factor responsible for regulating cambial divisions, based on a *pxy* loss-of-function phenotype and recent evidence suggesting that ethylene response factors that include ERF109 and ERF018 are expressed in procambial cells and act in a parallel pathway to control cell divisions (Etchells *et al*., 2012).

By similarity to the signalling pathways in the SAM and QC, the WOX transcription factors were likely able to regulate procambium cell division. Indeed, WOX4 and WOX14 function downstream of CLE41/TDIF and PXY/TDR (Hirakawa *et al*., 2010; Etchells *et al*., 2013). An additional hormonal input is provided by the auxin-dependent activation of WOX4, linking long-distance signalling with cell division in this niche (Suer *et al*., 2011). WOX4 and WOX14 regulate the number of cell divisions but not their orientation or vascular patterning (Ji *et al*., 2010; Etchells *et al*., 2013). Hence, orientation is regulated by a downstream target of PXY/TDR other than WOX4 or WOX14. Additionally, as with stomatal asymmetric cell divisions, the phloem-expressed receptor kinase ERECTA has been shown to have a role in the orientation of the procambial divisions (Etchells *et al*., 2013). ERECTA interacts with the cysteine-rich peptides EPFL4/CHALLAH-LIKE2 and EPFL6/CHALLAH secreted from the endodermis (Uchida and Tasaka, 2013), but as ERECTA is expressed in phloem and PXY/TDR in procambium and cambium, the interaction of their downstream pathways has not been deciphered so far.

Taken together, these findings indicate that the amplifying asymmetric divisions of procambium and cambium appear to be niche-controlled. A local signal, CLE41/42/44/TDIF, regulates both the orientation of cell division through unidentified targets and proliferation through WOX4 and WOX14. For the root procambium, there is evidence that a similar, if not the same, pathway might regulate these asymmetric cell divisions. Promoter studies (Hirakawa *et al*., 2008) and the root cell type-specific expression profiling data set (Brady *et al*., 2007) show that *PXY/TDR* is expressed in root procambium and root xylem, *CLE41* and *CLE44* in the root phloem and *WOX14* throughout the root. *WOX4* has also been shown to regulate root procambium proliferation in tomato (Ji *et al*., 2010).

. . . but many other signals affect (pro)cambium development

However, there are also other players in procambium and cambium development that have not been fully integrated with the model described above. These include DOF5·6/HIGH CAMBIAL ACTIVITY, a transcription factor likely to act in the initiation of the interfascicular cambium (Guo *et al*., 2009), XYLEM INTERMIXED WITH PHLOEM, an LRR-RLK that appears to prevent differentiation of the procambium and cambium (Bryan *et al*., 2012), the histidine-kinase CYTOKININ-INDEPENDENT1 and cytokinin receptors ARABIDOPSIS HISTIDINE KINASE2 (AHK2), AHK3 and AHK4, which are required for procambium proliferation (Mahonen *et al*., 2000; Hejatko *et al*., 2009).

The balance of HD-ZIP III transcription factors (REVOLUTA, PHABULOSA, PHAVOLUTA, CORONA, AtHB8) and KANADI transcription factors also affect procambium and cambium development. KANADIs act to inhibit PIN expression and auxin transport (Ilegems *et al*., 2010) and regulate procambium and cambium proliferation through regulation of WOX4. ATHB-8 is transcriptionally activated in pre-procambium by auxin via MONOPTEROS. ATHB-15 is expressed in a procambium-specific manner in arabidopsis and *Zinnia*, and popREVOLUTA and PtrHB7 are expressed in the cambium of *Populus* and regulate initiation of procambium and cambium activity during primary and secondary growth, respectively (Baima *et al*., 2001; Ohashi-Ito and Fukuda, 2003; Donner *et al*., 2009; Robischon *et al*., 2011; Zhu *et al*., 2013). It is apparent that the regulation of procambium and cambium proliferation and the identity and orientation of their divisions are mechanistically complex and that both long- and short-distance signals feed into the regulation.

To understand the regulation of procambium and cambium cell divisions and stem cell identity further, systems biology approaches have been adopted. As methods for isolating very rare cell types in arabidopsis have developed, different approaches have been taken to identify the transcripts upregulated in the cambium. First, large-scale transcript profiling studies for arabidopsis inflorescence stem vasculature used induction of secondary growth in vasculature by different methods, including application of increased weight (Ko *et al*., 2004) or exogenous auxin (Wenzel *et al*., 2008). More recently, a method combining induction of secondary growth by apical exogenous auxin application and laser capture microdissection identified two previously uncharacterized LRR-RLKs that regulate cambium activity, REDUCED IN LATERAL GROWTH1 (RUL1) and MORE LATERAL GROWTH1 (MOL1) (Agusti *et al*., 2011). *In silico* work to identify genes co-expressed with *PXY/TDR* and other transcripts known to accumulate in procambium and cambium also identified an LRR-RLK, PXY/TDR-CORRELATED1 (PXC1) (Wang *et al*., 2013). In order to start understanding how all these different inputs and players work together in vascular development, a model of interactions in arabidopsis procambium, phloem and xylem has been put together, allowing postulation of new interactions (Benitez and Hejatko, 2013). However, many of the interactions remain uncharacterized and our understanding of the regulation of procambium and cambium activity can be guided using other species with more extensive cambial tissue.

In woody species like trees, the vascular cambium gives rise to the great majority of the biomass in the form of wood. The original understanding of the role of auxin in vascular cambium was obtained from expression patterns of polar auxin transporters in hybrid aspen (*Populus tremula* × *tremuloides*) (Schrader *et al*., 2003). The transcriptome from *Populus tremula* stem tangential thin sections, including the cambium region, one of the first high spatial resolution transcriptome profiles in a non-arabidopsis species (Schrader *et al*., 2004). Vascular cambium regulators identified from hybrid aspen (*Populus tremula* × *alba*) include the *ARBORKNOX* genes, *PtaARK1* and

PtaARK2 (Groover *et al*., 2006; Du and Groover, 2010) and their regulator *LATERAL ORGAN BOUNDARIES DOMAIN*-family transcription factor *PtaLBD1* (Yordanov *et al*., 2010), which are not known to be involved in procambial or cambial development in arabidopsis.

Specification of asymmetric cell identity in the procambium and cambium: xylem versus phloem

The asymmetric cell division of procambium and cambium produces daughter cells that differentiate into either phloem or xylem. The identity of either phloem cell or xylem cell appears to be regulated by signals other than the PXY/TDR pathway. An LIM-domain protein, LATERAL ROOT DEVELOPMENT 3 (LRD3), promotes early phloem development (Ingram *et al*., 2011), an MYB transcription factor, ALTERED PHLOEM DEVELOPMENT (APL), functions in later stages of phloem differentiation and repression of xylem differentiation (Bonke *et al*., 2003; Truernit *et al*., 2008) and a membrane-associated protein, OCTOPUS (OPS), functions in the promotion of phloem continuity and protophloem differentiation (Truernit *et al*., 2012). Xylem cell specification and differentiation has been studied more comprehensively (Zhang *et al*., 2014). It is largely regulated by the Class III homeodomain leucine zipper (HD-ZIP III) transcription factors. Five HD-ZIP IIIs, PHABULOSA, PHAVOLUTA, REVOLUTA, CORONA/ATHB-15 and ATHB-8, are both necessary and sufficient for xylem cell specification and differentiation (Carlsbecker *et al*., 2010; Ilegems *et al*., 2010) and for radial patterning of the vasculature in conjunction with antagonistically acting KANADI transcription factors (Emery *et al*., 2003). Expression of the HD-ZIP IIIs is restricted to the vascular domain in roots by the mobile microRNA 165/166, from the endodermal layer, induced by SHR and SCR (Carlsbecker *et al*., 2010) and upregulated by externally applied brassinosteroids (Ohashi-Ito and Fukuda, 2003). With respect to asymmetric cell specification activity, the downstream specification of a daughter cell with procambium identity and with xylem identity is regulated in a complex manner by HD-ZIP III transcription factors. Many other transcription factors have been shown to regulate xylem cell specification, including the NAC domain transcription factors VND6 and VND7 and their downstream targets (extensively reviewed in Lucas *et al*., 2013*b*).

Completing our understanding of asymmetric divisions in procambial and cambial cells

There remains much room for traditional genetic, biochemical and systems biology approaches to guide our mechanistic understanding of asymmetric cell division activity in the procambium and cambium. Specifically, a major missing piece of knowledge concerns the positional influences that determine either xylem identity or phloem identity. Mapping the expression and protein profiles of these procambium and cambium cells over different developmental time points to determine the cues that influence division and asymmetric identity at cell type-resolution is greatly needed. Furthermore, protein–protein and protein–DNA interactions of the identified signalling pathway components could expand our knowledge of the pathway and elucidate how cell polarity, the cell proliferation rate and daughter cell identity are regulated on a molecular level. Also, resolving the microRNA, transcript and protein accumulation, epigenetic modification and hormone activity profiles in fine cell type-specific detail would allow more accurate modelling of vascular development.

STOMATAL DEVELOPMENT IN ARABIDOPSIS AND MAIZE: ASYMMETRIC DIVISIONS IN DISPERSED POPULATIONS

Developmentally important asymmetric divisions can also occur outside of organized niches. One such example is in the stomatal lineage of the aerial epidermis. Stomata serve as adjustable valves though which carbon dioxide (CO_2) from the atmosphere and water vapour from the plant interior are exchanged. Each stoma consists of a pair of sister epidermal cells (guard cells, GCs) around a central pore. Both the pattern and the fate specification of the guard cells (and their precursors) are associated with asymmetric and oriented divisions.

Recent evolutionary studies indicate that asymmetric divisions are associated with the stomata in many angiosperm lineages (Rudall *et al*., 2013), though molecular studies are so far restricted to two major groups of flowering plants: monocots, for which the grasses maize and rice serve as representatives, and dicots, for which arabidopsis is the primary model. Recent data from these systems point to models in which expression of cell type-specific transcription factors is guided by extensive local cell–cell signalling in regulating asymmetric divisions (Pillitteri and Torii, 2012). Moreover, these transcription factors and signals are required for the products of the asymmetric divisions to be correctly specified and arranged in a functionally adaptive pattern. However, there is also evidence that pre-divisional (intrinsic) cell polarities must be established for the stomatal asymmetric divisions, and several polarly localized proteins have been identified. To connect with previous tissue discussions, we will begin with monocot stomatal development because its spatiotemporal gradients bear superficial similarity to the situation in the root cortex/endodermis initial asymmetric divisions, and then move on to arabidopsis development, in which the asymmetric divisions represent a departure from the other systems.

In grasses, stomatal development proceeds in a well-defined gradient wherein the distal tip of the leaf bears the oldest and most mature cells and the base contains the youngest cells (Nelson and Langdale, 1992). Early asymmetric divisions are abundant in the proximal portions of the leaf (base) and differentiation proceeds in an orderly fashion as the distal tip is approached (Fig. 6A). Within these general zones, guard mother cells (GMCs, the immediate precursor cell type of the stomatal guard cells) are generated in specific cell files by asymmetric cell divisions. In the epidermis, all divisions are anticlinal, but the GMC-generating divisions are also all oriented in the same direction relative to the base–tip axis. In grasses, stomatal complexes consist of four cells: the GCs immediately surrounding the stomatal pore and two subsidiary cells on either side of the GCs (Fig. 6A). To generate this unit, the GMC sends a polarizing signal to its lateral neighbours. The neighbours become subsidiary mother cells (SMCs) and the interaction between GMC and SMC results in movement of the SMC nucleus towards the site of GMC contact, followed by a highly asymmetric division

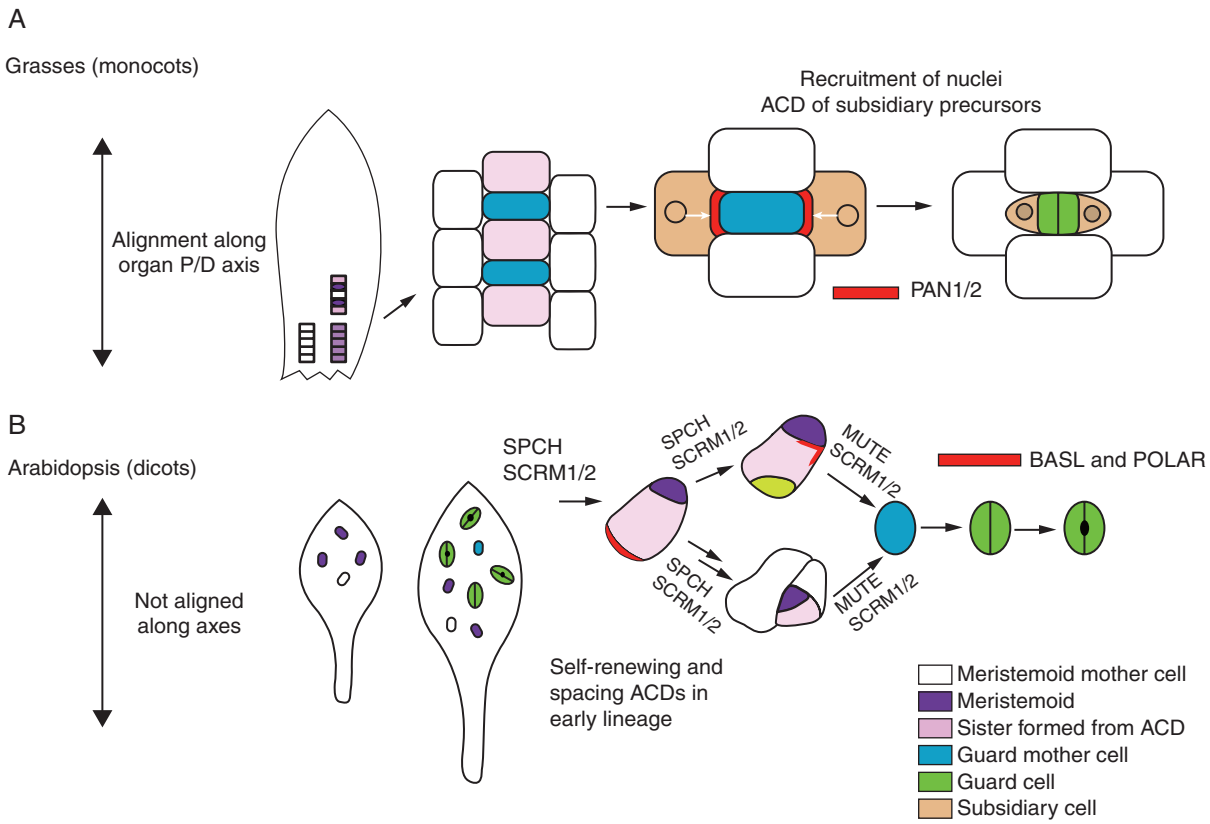

FIG. 6. General schemes for asymmetric divisions (ACD) in stomatal development. (A) Stomatal development in the grasses. Cells are arranged in linear files along the length of the leaves. In alternate files at the base of the leaf, asymmetric divisions generate guard mother cells (GMCs, blue). GMCs signal to the neighbouring cell files, inducing the migration of subsidiary mother cell (SMC) nuclei towards the point of contact with the GMCs and the subsequent asymmetric and oriented cell division of the SMCs to form the subsidiary cells. This is facilitated by the polarized proteins PAN1 and PAN2 (yellow lines). (B) In dicots such as arabidopsis, stomatal precursors are not arranged in files and an additional step precedes the formation of GMCs. The initiation of the stomatal lineage is an asymmetric division of a meristemoid mother cell (MMC, white) to produce a meristemoid (green) and a stomatal lineage ground cell (SLGC). A meristemoid can divide again in an 'amplifying division', renewing itself and producing another SLGC (lower pathway) and an SLGC can divide to create another meristemoid in a 'spacing division' (upper pathway). These divisions require the transcription factors SPCH, SCRM1 and SCRM2. There is evidence for intrinsic polarization of proteins such as BASL and POLAR (red lines) during both amplifying and spacing divisions. Meristemoids derived from either amplifying or spacing divisions will eventually differentiate into GMCs (blue; dependent on MUTE, SCRM1 and SCRM2) that later undergo a single symmetric division to form guard cells (purple). In arabidopsis, there are no cells equivalent to the subsidiary cells in the grasses.

of the SMC (Fig. 6A) to carve off a small subsidiary cell (SC). The GMC then divides symmetrically to form the proper guard cells and the whole complex undergoes radical cell morphology changes to produce a final functional unit (Bergmann and Sack, 2007).

Thus far, genes required for the asymmetric divisions that that specify GMC cell identity have not been identified. Two LRR-RLKs, *PANGLOSS1* (*PAN1*) and *PAN2*, however, have been shown to be required for two aspects of the SMC asymmetric division, specifically the orientation of SMC divisions and the specification of SC cell fate. Mutations in either *PAN1* or *PAN2* lead to defects in the alignment of the SMC nucleus with the cell edge that faces the GMC. This alignment failure results in abnormal cell division orientation and defects in subsidiary cell identity and shape (Gallagher and Smith, 2000; Cartwright *et al*., 2009; Zhang *et al*., 2012). PAN1 and PAN2 proteins accumulate in strikingly polarized sites at the SMC periphery corresponding to where the SMCs contact the GMC (Cartwright *et al*., 2009; Zhang *et al*., 2012) (Fig. 6A) and *PAN2* is genetically upstream of *PAN1* for this localization (Zhang *et al*., 2012). ROP family GTPases appear to be downstream effectors of the PANs, as they have been shown to accumulate at the GMC-adjacent site in a PAN-dependent manner and are also required for proper asymmetric divisions (Humphries *et al*., 2011).

Based on observations of nuclear and cytoskeletal movement and PAN protein localization prior to and during the asymmetric divisions, it appears that an extrinsic signal from the GMC is responsible for generating an intrinsic asymmetry in the SMC. An attractive model is that after signal-guided localization of the PANs, their activity (mediated via ROPs) organizes the SMC cytoskeleton and recruits the SMC nucleus. There are, however, some complications in this explanation. First, the cue from the GMC that positions PAN1 and PAN2 is unknown, and these proteins are never visible except in their polarized position (i.e. they are not first uniformly present at the plasma membrane and then concentrated at the GMC site), so it is unclear how early in a polarity hierarchy these proteins act. Secondly, although predicted to be kinases, PAN1 and PAN2 are either missing an essential residue for kinase activity and/or not able to phosphorylate themselves or a generic substrate *in vitro*

(Cartwright *et al*., 2009; Zhang *et al*., 2012). Identification of interaction partners of the PAN proteins is likely to be a very productive future direction.

Arabidopsis stomatal development

In maize, the linear arrangement of mature stomata is predicted by the linear arrangement of cell files and the asymmetric cell divisions within them. No such early predictive pattern exists in arabidopsis leaves. Instead, arabidopsis stomatal precursors are the products of divisions in a dispersed and apparently randomly selected group of cells in the immature epidermis (Fig. 6B). These initial cells in the pathway [the so called meristemoid mother cells (MMCs)] are not yet associated with any specific gene expression profile or location, so they are defined retrospectively. Asymmetric division of the MMC creates two differently sized daughters, a smaller meristemoid and a larger stomatal lineage ground cell (SLGC) (Fig. 6B). Both daughters can undergo additional asymmetric divisions, but the nature and cell fate outcome of these divisions differs. The meristemoid can undergo asymmetric 'amplifying' divisions (Bergmann and Sack, 2007), with the smaller daughter of each division round retaining meristemoid identity and the larger becoming an SLGC. An SLGC may differentiate into a large, lobed, pavement cell, or may become an MMC, dividing asymmetrically in a 'spacing division' to create a secondary meristemoid (Fig. 6B). The spacing divisions of SLGCs are so named because they are not only asymmetric, but are also oriented such that there is always at least one epidermal cell placed between a newly formed meristemoid and previously specified stomata or GMCs. Regardless of whether a meristemoid was produced via amplifying or spacing divisions, it will eventually differentiate into a GMC that undergoes a single symmetric division to produce the paired guard cells of the stoma.

In arabidopsis spacing divisions, cell–cell signalling plays a key role. Several receptors and receptor like-kinases have been implicated: the LRR receptor-like protein TOO MANY MOUTHS (TMM) was the first to be identified (Nadeau and Sack, 2002) and subsequently the LRR-RLKs ERECTA, ERECTA-LIKE1 and ERECTA-LIKE2 [collectively referred to as the ERECTA family (ERf)] were shown to mediate stomatal signalling through direct interactions with TMM (Shpak *et al*., 2005; Lee *et al*., 2012). Loss of TMM or ERf function results in mispatterned and excessive numbers of stomata, suggesting that the kinases orient asymmetric division and repress stomatal fate at various stages of lineage progression. Although TMM and the ERf belong to the same broad receptor class as maize PAN1/2, the proteins are not close relatives, nor has polarized subcellular localization been reported for TMM or the ERf (Nadeau and Sack, 2002; Lee *et al*., 2012). Additionally, ERECTA does appear to be an active kinase (Lease *et al*., 2001). Upstream of these receptors, a number of small, secreted peptides of the *EPIDERMAL PATTERNING FACTOR LIKE (EPFL)* family, appear to be the guiding cues. The founding member of the family, *EPF1* (Hara *et al*., 2007), and its closest homologue *EPF2* (Hara *et al*., 2009; Hunt and Gray, 2009) are both expressed specifically in stomatal lineage cells and both repress stomatal production, though they appear to act at slightly different stages and are ligands for different ERECTA receptor family members (Lee *et al*., 2012). Because the ligand and receptors are expressed in overlapping cells in the epidermis, one question that emerges is whether a single cell responds to the EPF signal it generates (an intrinsic or autocrine signal), or whether stomatal lineage cells block their own signals and respond only to those from their neighbours (an extrinsic or paracrine signal). Interestingly, a similar issue arises in mammalian epidermis, where Wnt signalling is responsible for regulating stem-cell like divisions and Wnt signals are both created and perceived by the same cell types (Habib *et al*., 2013). It is also difficult to uncouple a role for the ligands and receptors in orienting cell divisions versus regulating cell division rates, as ligand overexpression limits cell numbers, thus indirectly preventing misoriented divisions. Given the complexity in the EPF/ERf pathway and recent evidence for cross-talk with other signalling pathways (Gudesblat *et al*., 2012; Kim *et al*., 2012), systemic analysis of ligand and receptor expression pattern and binding properties (Lee *et al*., 2012) combined with modelling approaches to estimate the contribution of specific ligand–receptor (and higher order receptor-receptor) combinations to individual events (Ruiz-Herrero *et al*., 2013) will be needed.

As in the embryo and root, transcription factors play a critical role in asymmetric division and cell fate establishment in the stomatal lineage (Lai *et al*., 2005; Ohashi-Ito and Bergmann, 2006; Kutter *et al*., 2007; MacAlister *et al*., 2007; Pillitteri *et al*., 2007; Kanaoka *et al*., 2008). When considering the genes whose disruption or overexpression yields the most dramatic phenotypes, the bHLH factors rise to prominence; here five bHLH transcription factors serve as major cell fate regulators in the stomatal lineage (Ohashi-Ito and Bergmann, 2006; MacAlister *et al*., 2007; Pillitteri *et al*., 2007; Kanaoka *et al*., 2008). Three of these five [SPEECHLESS (SPCH), MUTE and FAMA] are expressed in restricted cell types and regulate corresponding individual stages of lineage progression (MacAlister *et al*., 2007; Ohashi-Ito and Bergmann, 2007; Pillitteri *et al*., 2007). Two factors in particular, SPCH and MUTE, bookend the asymmetric division phase, with SPCH promoting initiation of asymmetric divisions (formation of MMCs) and MUTE terminating the self-renewing division phase of these cells.

The position of SPCH at the start of the lineage and its unique possession of a domain capable of being phospho-regulated by MAPK and brassinosteroid-related (BIN2) kinases (Lampard *et al*., 2008; Gudesblat *et al*., 2012; Kim *et al*., 2012) have made this protein a prime target of interest for asymmetric division. Without SPCH, asymmetric divisions are eliminated in the leaf epidermis, suggesting that SPCH is necessary for allowing specific types of division. The SPCH protein is expressed very early in leaf development, possibly in all cells, but in a wild-type leaf it is highly regulated such that, before an asymmetric division, it accumulates in the MMC nucleus and is then inherited by both daughters, but is rapidly extinguished in the SLGC. Following SPCH–GFP expression over many days, Robinson *et al*. (2011) were able to derive a model for pattern formation in a subset of stomatal lineage cells. This model suggested that the presence of SPCH protein permitted cells to divide before they had doubled in size. Small size is correlated with meristemoid identity, so by controlling where SPCH accumulates it is possible to control which cells are meristemoids (stem cells) and which differentiate into a slow or non-dividing epidermal cell. Amplifying division-based patterning is essentially a lineage-based mechanism – the history, but not the neighbours

of a cell determine its fate. This patterning mechanism cannot apply to all stomatal cells; for example, signalling from neighbours is a requirement for organizing spacing divisions. However, it does highlight one of the best examples of intrinsic asymmetry generation in plants. It also requires an intrinsic polarizing factor that can 'read' the history of previous divisions and orient new divisions.

The best candidate to be the polarizer is the novel, plant-specific protein BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) (Dong *et al*., 2009). *BASL* is expressed primarily in asymmetrically dividing stomatal lineage cells, and its genetic disruption leads to stomatal lineage divisions with reduced physical and marker expression asymmetry and with defects in their final identity. In *basl*, products of the defective asymmetric divisions will sometimes all become stomata, or all become non-stomatal epidermal cells. This suggests that BASL is not required for a specific cell identity, but rather for helping to enforce differences (Dong *et al*., 2009). It is not only its function but also its dynamic protein localization that links BASL to polarity generation. In cells about to undergo an asymmetric division, BASL is found in the nucleus, but begins to accumulate in a cortical crescent. The crescent is positioned such that it is reliably inherited by the larger daughter. After division, the smaller daughter may express BASL in the nucleus, whereas the larger has BASL both in the nucleus and at the cortex (Dong *et al*., 2009). Time-lapse experiments tracing BASL dynamics in single cells revealed two possible developmental trajectories for each daughter. The smaller (meristemoid) can become a GMC, losing nuclear BASL in the process, or it can divide again asymmetrically after first establishing a new cortical crescent. The larger (SLGC) can differentiate into a non-stomatal epidermal cell, losing nuclear BASL (but sometimes retaining the cortical crescent), or it can divide asymmetrically to form a secondary meristemoid, a fate that correlated with retention of both nuclear and cortical BASL. Production of a secondary meristemoid (via a spacing division) requires that the SLGC reorient its axis of polarity in order to maintain one-cell spacing. This reorientation is reflected in the cortical BASL crescent's relocation to the opposite side of the cell, ensuring that it is distal to the newly forming meristemoid. In signalling mutants such as *tmm*, where division orientations are disrupted, BASL still exhibits normal accumulation in nuclei and at the crescent, but the location of the crescent is incorrect, suggesting that stomatal lineage cells generate a polarity using a BASL-related mechanism, but that this intrinsic polarity can be reoriented upon external cues (Dong *et al*., 2009). Such a situation is reminiscent of Wnt signalling in animal embryos, where a signal from a neighbour can reorient the division plane of an asymmetric division (Goldstein *et al*., 2006).

While BASL localization is intriguing, its lack of functional domains has made its precise function in asymmetric divisions difficult to ascertain. The model of Robinson *et al*. (2011) predicts that BASL would have to work pre-divisionally to guide the nucleus to an asymmetric location, thus acting intrinsically to specify asymmetry. Overexpression of BASL can lead to localized outgrowths, leading to another model, in which BASL is required post-divisionally for expanding the SLGC cell (Dong *et al*., 2009). This latter phenotype is dependent on normal ROP GTPase signalling, suggesting a potential commonality between the division orientation of the SMCs of the maize stomatal complexes and the divisions of the stomatal MMCs. A greater understanding of the mechanics of polarization is needed. While classical cell biology may answer this question, transcriptional profiling of the stomatal lineage can also add to the repertoire of polarity factors, as shown recently for another novel protein, POLAR (Pillitteri *et al*., 2011).

PERSPECTIVES

Similarities in molecular mechanisms of extrinsic asymmetric divisions amongst plant cells

Cell division and expansion-based morphogenesis necessitated by encasement in rigid walls means that plant cells are in predictable and long-term spatial relationships with their neighbours. Because of this, it is theoretically possible for most formative divisions to rely only on external (signalling) control of asymmetric divisions. Among the extrinsically regulated asymmetric divisions discussed throughout this review, the present data support multiple common regulatory mechanisms, including small peptide/receptor-like kinase signalling, hormone signalling, mobile transcription factors and nuclear migration. Many molecule classes are similar in different cell populations, suggesting general mechanisms by which asymmetric cell divisions can be regulated in plants. RLKs have been described to function in lateral root initiation (ACR4), procambium and cambium development (PXY) and stomatal development (PAN1/PAN2, ERf). These molecules are membrane-bound and are thus positioned appropriately as mediators of extrinsic signalling. The exact peptide(s) responsible for signalling have been identified for procambium and cambium signalling (CLE41/42 and 44) and for arabidopsis stomata (EPF1/2), but they remain to be identified for the remaining receptors. Conversely, CLEL6 and CLEL7 are small peptides that play a role in later asymmetric divisions within lateral root development, although their corresponding LRR-RLK has not been identified (Meng *et al*., 2012). The ERECTA LRR-RLK influences both (pro)cambial and stomatal development, demonstrating one case where a single receptor can regulate asymmetric cell division in multiple cell types (Shpak *et al*., 2005; Lee *et al*., 2012; Etchells *et al*., 2013). Perhaps this conservation of function is due to the fact that these are examples of asymmetric amplifying divisions.

Mobile transcription factors are of particular importance in root development. The TMO7 bHLH transcription factor protein is produced in the apical cells of the embryo, but moves into the basal hypophysis cell to regulate its asymmetric division (Schlereth *et al*., 2010). SHR, a GRAS-family transcription factor protein, moves from vascular tissue into the cortex/endodermis initial to regulate its asymmetric division (Nakajima *et al*., 2001). Evidence of the intricate dynamics of mobile factor regulation is further provided by the regulation of microRNA 165/166, which is directly transcriptionally downstream of SHR and its partner SCR (Carlsbecker *et al*., 2010; Miyashima *et al*., 2011). microRNA 165/166 diffuses in a gradient into the vasculature, thereby regulating levels of the HD-ZIP III factors, which then regulate asymmetric divisions of procambial cells and xylem identity.

Hormones provide an alternative regulatory mechanism. Auxin is essential for multiple formative asymmetric divisions within the embryo, including the generation of procambial cell

files (De Rybel *et al*., 2013), the hypophysis division that gives rise to the QC and the columella initial cell (Schlereth *et al*., 2010) and in the adult plant, including the cortex/endodermis initial (Cruz-Ramirez *et al*., 2012), procambial and cambial cells (Suer *et al*., 2011) and multiple formative divisions within the lateral root (De Smet *et al*., 2007; Dubrovsky *et al*., 2008, 2011; Parizot *et al*., 2008; Moreno-Risueno *et al*., 2010; Bishopp *et al*., 2011). One classic example of the generation of a niche is by targeted auxin flux from endodermis cells to neighbouring pericycle cells in order to specify the founder cell that will eventually form the lateral root primordia (Marhavy *et al*., 2013). In stomatal precursor divisions, PIN proteins and auxin signalling components regulate cell size and fate asymmetries as well as the number of precursor cell divisions (Le *et al*., 2014). Cytokinin is also becoming implicated in many of these processes. Cytokinin negatively influences lateral root priming, lateral root founder cell specification and the first asymmetric division of founder cells (Li *et al*., 2006; Laplaze *et al*., 2007; Chang *et al*., 2013). In some cases, cytokinin acts by opposing auxin through repression of PIN proteins. Cytokinin plays a primary role in promoting procambial cell proliferation, with mutations in the cytokinin receptor resulting in greatly reduced numbers of procambial cells and thereby fewer vascular cells (Mahonen *et al*., 2000).

Recent advances in high-resolution, real-time microscopy have revealed that nuclear migration plays a role in two formative asymmetric divisions – the first asymmetric division of pericycle cells and stomatal cell development – and, given the connections between nuclear position and pre-prophase band placement (Facette and Smith, 2012), it is likely that any physically asymmetric division will involve pre-divisional nuclear and organellar migration. Two distinct classes of molecules have been shown to regulate migrations. In lateral roots, the SLR/IAA14–ARF7–ARF19 module regulates the migration of founder cell nuclei towards their common cell wall just prior to lateral root initiation (De Smet *et al*., 2007; Dubrovsky *et al*., 2008; De Rybel *et al*., 2010; Goh *et al*., 2012). In the case of maize stomatal cells, GMCs signal to neighbouring cell files and induce the migration of a subsidiary mother cell nuclei towards the GMC cell, a process that requires the RLKs PAN1 and PAN2 (Gallagher and Smith, 2000; Cartwright *et al*., 2009; Zhang *et al*., 2012). So, although nuclear migration is a common mechanism that appears to signal asymmetric cell division, the molecular regulators known thus far belong to different classes.

Are there other classes of molecules that may extrinsically regulate asymmetric cell divisions but which have not been characterized? A novel non-auxin molecule, naxillin, has been used to characterize the role of an auxin precursor in lateral root initiation (De Rybel *et al*., 2012). Cytosolic Ca^{2+} is known as a classic signalling molecule and changes in the cytosolic Ca^{2+} level have been observed in roots upon induction of a mechanical stress. Blocking of the calcium channel impedes both changes in cytosolic Ca^{2+} and the production of lateral root primordia after bending, suggesting involvement of Ca^{2+} signalling in translation of the mechanical forces to a developmental response in lateral root primordium development (Richter *et al*., 2009; Toyota and Gilroy, 2013; Laskowski, 2013). Furthermore, recent studies suggest that callose-dependent plasmodesmatal symplastic transport plays a role in lateral root initiation and distribution. The activity of two callose-degrading enzymes, β-1,3-glucanase, plasmodesma-localized β-1,3 glucanase 1 (PdBG1) and PdBG2, affects symplastic connectivity, which is indicated by the *pdbg1,2* double mutants exhibiting restricted plasmodesmatal transport, increased lateral root density and distorted primordium patterning (Benitez-Alfonso *et al*., 2013; Vanstraelen and Beeckman, 2013). In the future, even more players are likely to be identified that establish distinct niches that control asymmetric cell divisions.

Elucidation of intrinsic molecular mechanisms

Intrinsic mechanisms are certainly less well characterized than extrinsic ones in plant cells. Potential exceptions to extrinsic control are seen in the early divisions of the arabidopsis stomatal lineage, where mechanisms are needed to distinguish between the two daughters of an asymmetric division without reference to any obvious external constant (such as proximity to specific landmarks or alignment along growth axes). In these cells, BASL and POLAR proteins mark restricted areas of the cell periphery before division, indicating that these cells have the intrinsic ability to move components to regions that would be differentially inherited. Paradoxically, however, although analysis of BASL and POLAR revealed this polarization capacity, neither BASL nor POLAR is truly differentially segregated: a nuclear pool of BASL is inherited by both daughters (Dong *et al*., 2009) and POLAR is enriched but not exclusively present on one side of the cell (Pillitteri *et al*., 2011). In both cases, it is the post-divisional behaviours of the proteins that show clear differences between sisters. While these sister cell differences could be amplifications of programmes started by differential segregation of components, they could equally be explained by post-divisional communication between the sister cells. Likewise, the 'master regulator' activities of SPCH and MUTE have made it appealing to consider them as segregated determinants, but the details of their expression patterns make this unlikely. When SPCH or MUTE expressing cells divide, both daughters inherit maternal proteins; it is differential maintenance of the proteins that distinguish the daughters (MacAlister *et al*., 2007; Pillitteri *et al*., 2007, 2008; Lampard *et al*., 2008; Robinson *et al*., 2011).

Other examples of intrinsic control can be hypothesized based on available data. For instance, auxin is polarly transported both into and out of a cell, and thus an auxin gradient can be established within a cell and could guide asymmetric cell divisions. An auxin maximum within lateral root founder cells is certainly required for an asymmetric division to take place (Geldner *et al*., 2004; Laskowski *et al*., 2008; Okumura *et al*., 2013). During embryogenesis, the egg cell undergoes two polarization steps, and these steps may require segregating determinants. Single-cell measurements of protein, transcript and small RNA abundance will be required to determine whether such molecules do exhibit segregation within the cell that leads to division and differential identity acquisition.

Remaining questions regarding plant asymmetric divisions

While characterizing the molecular mechanisms regulating asymmetric cell divisions, novel questions have been generated and still others remain to be answered. First, with respect to signals that are required to initiate cell division, focused

co-accumulation of signalling factors have been identified. Examples include TMO7/LHW, whose co-localization promotes embryonic vascular cell asymmetric divisions (De Rybel *et al*., 2013). Conversely, RBR and SCR are localized within the same cell and their physical interaction *represses* asymmetric cell division within the cortex/endodermis initial (Cruz-Ramirez *et al*., 2012). Phosphorylation via CYCD6;1 inhibits the RBR–SCR interaction and permits division (Cruz-Ramirez *et al*., 2012). Further, adding complexity to this story, auxin promotes CYCD6;1 accumulation and JACKDAW negatively controls the extent of asymmetric divisions (Welch *et al*., 2007). Therefore, a full characterization of the initiation of asymmetric divisions must encompass an inventory of proteins that could participate in protein complexes that limit as well as promote the number of asymmetric cell divisions.

The stereotyped orientation of the cell division plane in an asymmetric division furthermore appears to not be a generalizable rule across plant cells. For instance, in lateral root primordia the first few divisions are required to be stereotyped in their orientation (and the AUR kinases have been shown to regulate this orientation), but later divisions do not need to adhere to this rule in order for normal lateral root morphogenesis to occur (De Smet *et al*., 2008). The plane of division appears to be of great importance in cambial divisions in order to generate concentric cylinders of xylem, cambium and phloem files, since mutations in the PXY/TDR receptor result in a disorganized pattern; however, the mechanism by which the organization occurs is still unknown, as is the mechanism by which xylem and phloem are found on opposing sides of the procambium (Fisher and Turner, 2007). In the arabidopsis embryo, highly regular division patterns occur, while in other plant species the divisions can appear morphologically chaotic, suggesting that, at least evolutionarily, stereotyped organization may be dispensable. Furthermore, with respect to the cortex/endodermis initial, defects in divisions of this cell's embryonic precursors can be rescued after embryogenesis by unknown mechanisms, again suggesting that orientation can be dispensable (Sozzani *et al*., 2010). Addressing these questions in each cell type will require tools to uncouple cell division orientation from factors that specify distinct cell identities.

Network dynamics and the role of cell type-specific data acquisition

Feedback and feedforward regulation add additional complexity and precision to the regulation of these extremely important cell divisions during plant development. In the case of the cortex/endodermis initial and stomatal cells, modelling approaches have proven essential to elucidate these mechanisms (Robinson *et al*., 2011; Cruz-Ramirez *et al*., 2012). In the future, more comprehensive mapping of all factors that play a role in asymmetric cell divisions will be needed, as will mechanisms to carefully measure the dynamics of these interactions at cell-type resolution. Application of such computational approaches to the acquisition of data with high spatial and temporal resolution will likely provide answers to some of these remaining questions regarding the asymmetric divisions of plant cells.

ACKNOWLEDGEMENTS

K.K. is funded by a Finnish Cultural Foundation fellowship. P.R. was supported through a University of Nottingham Vice Chancellor Scholarship for Research Excellence. Work on asymmetric divisions in D.C.B.'s laboratory was funded by US National Science Foundation grant IOS-0845521. D.C.B. is a Gordon and Betty Moore Foundation investigator of the Howard Hughes Medical Institute. This work was supported by a Biotechnology and Biological Science Research Council (BBSRC) US Partnering Award (BB/J020117/1), a BBSRC David Phillips Fellowship (BB_BB/H022457/1) and a Marie Curie European Reintegration grant (PERG06-GA-2009-256354) to I.D.S. Work in R.S.'s laboratory is funded by the North Carolina Agricultural Research Service and North Carolina State University Provost's Office. D.W. would like to thank Bert De Rybel and Jos Wendrich for help with the figure and all members of his team for inspiring discussions. Work on asymmetric cell division in D.W.'s laboratory is funded by the European Research Council (ERC; CELLPATTERN contract 281573) and the Netherlands Organisation for Scientific Research (NWO; ALW grants 846.11.001 and 820.02.019). S.M.B. was funded for this work by a Katherine Esau junior faculty fellowship. S.M.B. and K.K. would like to thank Peter Etchells for helpful discussions and Fig. 5B.

LITERATURE CITED

Agusti J, Lichtenberger R, Schwarz M, Nehlin L, Greb T. 2011. Characterization of transcriptome remodeling during cambium formation identifies MOL1 and RUL1 as opposing regulators of secondary growth. *PLoS Genetics* **7**: e1001312.

Autran D, Baroux C, Raissig MT, *et al*. 2011. Maternal epigenetic pathways control parental contributions to *Arabidopsis* early embryogenesis. *Cell* **145**: 707–719.

Baima S, Possenti M, Matteucci A, *et al*. 2001. The arabidopsis ATHB-8 HD-zip protein acts as a differentiation-promoting transcription factor of the vascular meristems. *Plant Physiology* **126**: 643–655.

Baum SF, Dubrovsky JG, Rost TL. 2002. Apical organization and maturation of the cortex and vascular cylinder in *Arabidopsis thaliana* (Brassicaceae) roots. *American Journal of Botany* **89**: 908–920.

Beeckman T, Burssens S, Inzé D. 2001. The peri-cell-cycle in *Arabidopsis*. *Journal of Experimental Botany* **52**: 403–411.

Belmonte MF, Kirkbride RC, Stone SL, *et al*. 2013. Comprehensive developmental profiles of gene activity in regions and subregions of the *Arabidopsis* seed. Proceedings of the National Academy of Sciences of the USA **110**: E435–E444.

Benfey PN, Linstead PJ, Roberts K, Schiefelbein JW, Hauser MT, Aeschbacher RA. 1993. Root development in *Arabidopsis*: four mutants with dramatically altered root morphogenesis. *Development* **119**: 57–70.

Benitez M, Hejatko J. 2013. Dynamics of cell-fate determination and patterning in the vascular bundles of *Arabidopsis thaliana*. *PLoS One* **8**: e63108.

Benitez-Alfonso Y, Faulkner C, Pendle A, Miyashima S, Helariutta Y, Maule A. 2013. Symplastic intercellular connectivity regulates lateral root patterning. *Developmental Cell* **26**: 136–147.

Benková E, Michniewicz M, Sauer M, *et al*. 2003. Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115**: 591–602.

Benková E, Ivanchenko MG, Friml J, Shishkova S, Dubrovsky JG. 2009. A morphogenetic trigger: is there an emerging concept in plant developmental biology? *Trends in Plant Science* **14**: 189–193.

Berckmans B, Vassileva V, Schmid SP, *et al*. 2011. Auxin-dependent cell cycle reactivation through transcriptional regulation of *Arabidopsis* E2Fa by lateral organ boundary proteins. *Plant Cell* **23**: 3671–3683.

van den Berg C, Willemsen V, Hage W, Weisbeek P, Scheres B. 1995. Cell fate in the *Arabidopsis* root-meristem determined by directional signaling. *Nature* **378**: 62–65.

van den Berg C, Willemsen V, Hendriks G, Weisbeek P, Scheres B. 1997. Short-range control of cell differentiation in the *Arabidopsis* root meristem. *Nature* **390**: 287–289.

Bergmann DC, Sack FD. 2007. Stomatal development. *Annual Review of Plant Biology* **58**: 163–181.

Betschinger J, Mechtler K, Knoblich JA. 2006. Asymmetric segregation of the tumor suppressor brat regulates self-renewal in *Drosophila* neural stem cells. *Cell* **124**: 1241–1253.

Bielach A, Podlešáková K, Marhavý P, *et al*. 2012. Spatiotemporal regulation of lateral root organogenesis in *Arabidopsis* by cytokinin. *Plant Cell Online* **24**: 3967–3981.

Birnbaum K, Shasha DE, Wang JY, *et al*. 2003. A gene expression map of the *Arabidopsis* root. *Science* **302**: 1956–1960.

Bishopp A, Help H, El-Showk S, *et al*. 2011. A mutually inhibitory interaction between auxin and cytokinin specifies vascular pattern in roots. *Current Biology* **21**: 917–926.

Bonke M, Thitamadee S, Mahonen AP, Hauser MT, Helariutta Y. 2003. APL regulates vascular tissue identity in *Arabidopsis*. *Nature*, **426**: 181–186.

Brady SM, Orlando DA, Lee JY, *et al*. 2007. A high-resolution root spatiotemporal map reveals dominant expression patterns. *Science* **318**: 801–806.

Breuninger H, Rikirsch E, Hermann M, Ueda M, Laux T. 2008. Differential expression of WOX genes mediates apical-basal axis formation in the *Arabidopsis* embryo. *Developmental Cell* **14**: 867–876.

Bruex A, Kainkaryam RM, Wieckowski Y, *et al*. 2012. A gene regulatory network for root epidermis cell differentiation in *Arabidopsis*. *PLoS Genetics* **8**: e1002446.

Bryan AC, Obaidi A, Wierzba M, Tax FE. 2012. XYLEM INTERMIXED WITH PHLOEM1, a leucine-rich repeat receptor-like kinase required for stem growth and vascular development in Arabidopsis thaliana. *Planta* **235**: 111–122.

Carlsbecker A, Lee JY, Roberts CJ, *et al*. 2010. Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* **465**: 316–321.

Cartwright HN, Humphries JA, Smith LG. 2009. PAN1: a receptor-like protein that promotes polarization of an asymmetric cell division in maize. *Science* **323**: 649–651.

Casimiro I, Marchant A, Bhalerao RP, *et al*. 2001. Auxin transport promotes *Arabidopsis* lateral root initiation. *Plant Cell* **13**: 843–852.

Casimiro I, Beeckman T, Graham N, *et al*. 2003. Dissecting *Arabidopsis* lateral root development. *Trends in Plant Science* **8**: 165–171.

Casson S, Spencer M, Walker K, Lindsey K. 2005. Laser capture microdissection for the analysis of gene expression during embryogenesis of *Arabidopsis*. *Plant Journal* **42**: 111–123.

Chang L, Ramireddy E, Schmülling T. 2013. Lateral root formation and growth of *Arabidopsis* is redundantly regulated by cytokinin metabolism and signalling genes. *Journal of Experimental Botany* **64**: 5021–5032.

Clark SC, Kamen R. 1987. The human hematopoietic colony-stimulating factors. *Science* **236**: 1229–1237.

Clark SE, Williams RW, Meyerowitz EM. 1997. The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis*. *Cell* **89**: 575–585.

Cordes KR, Sheehy NT, White MP, *et al*. 2009. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature* **460**: 705–710.

Cosgrove DJ. 2005. Growth of the plant cell wall. *Nature Reviews. Molecular Cell Biology* **6**: 850–861.

Cruz-Ramirez A, Diaz-Trivino S, Blilou I, *et al*. 2012. A bistable circuit involving SCARECROW-RETINOBLASTOMA integrates cues to inform asymmetric stem cell division. *Cell* **150**: 1002–1015.

Cruz-Ramírez A, Díaz-Triviño S, Wachsman G, *et al*. 2013. A SCARECROW-RETINOBLASTOMA protein network controls protective quiescence in the arabidopsis root stem cell organizer. *PLoS Biology* **11**: e1001724.

Cui H, Levesque MP, Vernoux T, *et al*. 2007. An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants. *Science* **316**: 421–425.

Van Damme D, De Rybel B, Gudesblat G, *et al*. 2011. *Arabidopsis* α AURORA kinases function in formative cell division plane orientation. *Plant Cell* **23**: 4013–4024.

Deal RB, Henikoff S. 2010. A simple method for gene expression and chromatin profiling of individual cell types within a tissue. *Developmental Cell* **18**: 1030–1040.

DiDonato RJ, Arbuckle E, Buker S, *et al*. 2004. *Arabidopsis* ALF4 encodes a nuclear-localized protein required for lateral root formation. *Plant Journal* **37**: 340–353.

DiLaurenzio L, WysockaDiller J, Malamy JE, *et al*. 1996. The SCARECROW gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root. *Cell* **86**: 423–433.

Ditengou FA, Tealea WD, Kochersperger P, *et al*. 2008. Mechanical induction of lateral root initiation in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences of the USA **105**: 18818–18823.

Dong J, MacAlister CA, Bergmann DC. 2009. BASL controls asymmetric cell division in *Arabidopsis*. *Cell* **137**: 1320–1330.

Donner TJ, Sherr I, Scarpella E. 2009. Regulation of preprocambial cell state acquisition by auxin signaling in *Arabidopsis* leaves. *Development* **136**: 3235–3246.

Du J, Groover A. 2010. Transcriptional regulation of secondary growth and wood formation. *Journal of Integrative Plant Biology* **52**: 17–27.

Dubrovsky J, Rost T, Colón-Carmona A, Doerner P. 2001. Early primordium morphogenesis during lateral root initiation in *Arabidopsis thaliana*. *Planta* **214**: 30–36.

Dubrovsky JG, Sauer M, Napsucialy-Mendivil S, *et al*. 2008. Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. Proceedings of the National Academy of Sciences of the USA **105**: 8790–8794.

Dubrovsky JG, Soukup A, Napsucialy-Mendivil S, Jeknic Z, Ivanchenko MG. 2009. The lateral root initiation index: an integrative measure of primordium formation. *Annals of Botany* **103**: 807–817.

Dubrovsky JG, Napsucialy-Mendivil S, Duclercq J, *et al*. 2011. Auxin minimum defines a developmental window for lateral root initiation. *New Phytologist* **191**: 970–983.

Emery JF, Floyd SK, Alvarez J, *et al*. 2003. Radial patterning of *Arabidopsis* shoots by class IIIHD-ZIP and KANADI genes. *Current Biology* **13**: 1768–1774.

Esau K. 1965. *Plant anatomy*, 2nd edn. New York: John Wiley.

Etchells JP, Turner SR. 2010. The PXY-CLE41 receptor ligand pair defines a multifunctional pathway that controls the rate and orientation of vascular cell division. *Development* **137**: 767–774.

Etchells JP, Provost CM, Turner SR. 2012. Plant vascular cell division is maintained by an interaction between PXY and ethylene signalling. *PLoS Genetics* **8**: e1002997.

Etchells JP, Provost CM, Mishra L, Turner SR. 2013. WOX4 and WOX14 act downstream of the PXY receptor kinase to regulate plant vascular proliferation independently of any role in vascular organisation. *Development* **140**: 2224–2234.

Facette MR, Smith LG. 2012. Division polarity in developing stomata. *Current Opinion in Plant Biology* **15**: 585–592.

Fernandez A, Drozdzecki A, Hoogewijs K, *et al*. 2013*a*. Transcriptional and functional classification of the GOLVEN/ROOT GROWTH FACTOR/CLE-like signaling peptides reveals their role in lateral root and hair formation. *Plant Physiology* **161**: 954–970.

Fernandez A, Hilson P, Beeckman T. 2013*b*. GOLVEN peptides as important regulatory signalling molecules of plant development. *Journal of Experimental Botany* **64**: 5281–5296.

Fisher K, Turner S. 2007. PXY, a receptor-like kinase essential for maintaining polarity during plant vascular-tissue development. *Current Biology* **17**: 1061–1066.

Fortin MC, Pierce FJ, Poff KL. 1989. The pattern of secondary root formation in curving roots of *Arabidopsis thaliana* (L.) Heynh. *Plant, Cell & Environment* **12**: 337–339.

Friml J, Vieten A, Sauer M, *et al*. 2003. Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. *Nature* **426**: 147–153.

Fukaki H, Tameda S, Masuda H, Tasaka M. 2002. Lateral root formation is blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of *Arabidopsis*. *Plant Journal* **29**: 153–168.

Fukaki H, Nakao Y, Okushima Y, Theologis A, Tasaka M. 2005. Tissue-specific expression of stabilized SOLITARY-ROOT/IAA14 alters lateral root development in *Arabidopsis*. *Plant Journal* **44**: 382–395.

Fuller MT, Spradling AC. 2007. Male and female *Drosophila* germline stem cells: two versions of immortality. *Science* **316**: 402–404.

Gallagher K, Smith LG. 2000. Roles for polarity and nuclear determinants in specifying daughter cell fates after an asymmetric cell division in the maize leaf. *Current Biology* **10**: 1229–1232.

Gallagher KL, Benfey PN. 2009. Both the conserved GRAS domain and nuclear localization are required for SHORT-ROOT movement. *Plant Journal* **57**: 785–797.

Gallagher KL, Paquette AJ, Nakajima K, Benfey PN. 2004. Mechanisms regulating SHORT-ROOT intercellular movement. *Current Biology* **14**: 1847–1851.

Gaudinier A, Zhang L, Reece-Hoyes JS, *et al*. 2011. Enhanced Y1H assays for *Arabidopsis*. *Nature Methods* **8**: 1053–1055.

Geldner N, Richter S, Vieten A, *et al.* 2004. Partial loss-of-function alleles reveal a role for GNOM in auxin transport-related, post-embryonic development of *Arabidopsis*. *Development* **131**: 389–400.

Goh T, Joi S, Mimura T, Fukaki H. 2012. The establishment of asymmetry in *Arabidopsis* lateral root founder cells is regulated by LBD16/ASL18 and related LBD/ASL proteins. *Development* **139**: 883–893.

Goldstein B, Takeshita H, Mizumoto K, Sawa H. 2006. Wnt signals can function as positional cues in establishing cell polarity. *Developmental Cell* **10**: 391–396.

Groover AT, Mansfield SD, DiFazio SP, *et al.* 2006. The *Populus* homeobox gene ARBORKNOX1 reveals overlapping mechanisms regulating the shoot apical meristem and the vascular cambium. *Plant Molecular Biology* **61**: 917–932.

Gudesblat GE, Schneider-Pizon J, Betti C, *et al.* 2012. SPEECHLESS integrates brassinosteroid and stomata signalling pathways. *Nature Cell Biology* **14**: 548–554.

Guo Y, Qin G, Gu H, Qu L-J. 2009. Dof5.6/HCA2, a Dof transcription factor gene, regulates interfascicular cambium formation and vascular tissue development in *Arabidopsis*. *Plant Cell* **21**: 3518–3534.

Habib SJ, Chen B-C, Tsai F-C, *et al.* 2013. A localized Wnt signal orients asymmetric stem cell division *in vitro*. *Science* **339**: 1445–1448.

Haecker A, Gross-Hardt R, Geiges B, *et al.* 2004. Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana*. *Development* **131**: 657–668.

Hamann T, Mayer U, Jurgens G. 1999. The auxin-insensitive bodenlos mutation affects primary root formation and apical-basal patterning in the *Arabidopsis* embryo. *Development* **126**: 1387–1395.

Hara K, Kajita R, Torii KU, Bergmann DC, Kakimoto T. 2007. The secretory peptide gene EPF1 enforces the stomatal one-cell-spacing rule. *Genes & Development* **21**: 1720–1725.

Hara K, Yokoo T, Kajita R, *et al.* 2009. Epidermal cell density is autoregulated via a secretory peptide, EPIDERMAL PATTERNING FACTOR 2 in *Arabidopsis* leaves. *Plant and Cell Physiology* **50**: 1019–1031.

Hardtke CS, Berleth T. 1998. The *Arabidopsis* gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO Journal* **17**: 1405–1411.

Heidstra R, Welch D, Scheres B. 2004. Mosaic analyses using marked activation and deletion clones dissect Arabidopsis SCARECROW action in asymmetric cell division. *Genes & Development* **18**: 1964–1969.

Hejatko J, Ryu H, Kim G-T, *et al.* 2009. The histidine kinases CYTOKININ-INDEPENDENT1 and ARABIDOPSIS HISTIDINE KINASE2 and 3 regulate vascular tissue development in *Arabidopsis* shoots. *Plant Cell* **21**: 2008–2021.

Helariutta Y, Fukaki H, Wysocka-Diller J, *et al.* 2000. The SHORT-ROOT gene controls radial patterning of the *Arabidopsis* root through radial signaling. *Cell* **101**: 555–567.

Himanen K, Boucheron E, Vanneste S, de Almeida Engler J, Inzé D, Beeckman T. 2002. Auxin-mediated cell cycle activation during early lateral root initiation. *Plant Cell* **14**: 2339–2351.

Hirakawa Y, Shinohara H, Kondo Y, *et al.* 2008. Non-cell-autonomous control of vascular stem cell fate by a CLE peptide/receptor system. Proceedings of the National Academy of Sciences of the USA **105**: 15208–15213.

Hirakawa Y, Kondo Y, Fukuda H. 2010. TDIF peptide signaling regulates vascular stem cell proliferation via the WOX4 homeobox gene in *Arabidopsis*. *Plant Cell* **22**: 2618–2629.

Hofhuis H, Laskowski M, Du Y, *et al.* 2013. Phyllotaxis and rhizotaxis in *Arabidopsis* are modified by three PLETHORA transcription factors. *Current Biology* **23**: 956–962.

Humphries JA, Vejlupkova Z, Luo A, *et al.* 2011. ROP GTPases act with the receptor-like protein PAN1 to polarize asymmetric cell division in maize. *Plant Cell* **23**: 2273–2284.

Hunt L, Gray JE. 2009. The signaling peptide EPF2 controls asymmetric cell divisions during stomatal development. *Current Biology* **19**: 864–869.

Ikeyama Y, Tasaka M, Fukaki H. 2010. RLF, a cytochrome b(5)-like heme/steroid binding domain protein, controls lateral root formation independently of ARF7/19-mediated auxin signaling in *Arabidopsis thaliana*. *Plant Journal* **62**: 865–875.

Ilegems M, Douet V, Meylan-Bettex M, *et al.* 2010. Interplay of auxin, KANADI and Class III HD-ZIP transcription factors in vascular tissue formation. *Development* **137**: 975–984.

Ingram P, Dettmer J, Helariutta Y, Malamy JE. 2011. *Arabidopsis* Lateral Root Development 3 is essential for early phloem development and function, and hence for normal root system development. *Plant Journal* **68**: 455–467.

Ito Y, Nakanomyo I, Motose H, *et al.* 2006. Dodeca-CLE peptides as suppressors of plant stem cell differentiation. *Science* **313**: 842–845.

Ji J, Strable J, Shimizu R, Koenig D, Sinha N, Scanlon MJ. 2010. WOX4 promotes procambial development. *Plant Physiology* **152**: 1346–1356.

Johri BM, Ambegaokar KB, Srivastava PS. 1992. *Comparative embryology of angiosperms*. Berlin: Springer.

Jurado S, Abraham Z, Manzano C, López-Torrejón G, Pacios LF, Del Pozo JC. 2010. The *Arabidopsis* cell cycle F-box protein SKP2A binds to auxin. *Plant Cell* **22**: 3891–3904.

Jurado S, Diaz-Trivino S, Abraham Z, Manzano C, Gutierrez C, del Pozo JC. 2008. SKP2A protein, an F-box that regulates cell division, is degraded via the ubiquitin pathway. *Plant Signaling & Behavior* **3**: 810–812.

Jürgens G, Mayer U. 1994. Arabidopsis. In: Bard JBL. ed. *Embryos. Colour atlas of development*. London: Wolfe Publishing.

Kanaoka MM, Pillitteri LJ, Fujii H, *et al.* 2008. SCREAM/ICE1 and SCREAM2 specify three cell-state transitional steps leading to *Arabidopsis* stomatal differentiation. *Plant Cell* **20**: 1775–1785.

Kang YH, Song S-K, Schiefelbein J, Lee MM. 2013. Nuclear trapping controls the position-dependent localization of CAPRICE in the root epidermis of *Arabidopsis*. *Plant Physiology* **163**: 193–204.

Kim TW, Michniewicz M, Bergmann DC, Wang ZY. 2012. Brassinosteroid regulates stomatal development by GSK3-mediated inhibition of a MAPK pathway. *Nature* **482**: 419–422.

Ko JH, Han KH, Park S, Yang JM. 2004. Plant body weight-induced secondary growth in *Arabidopsis* and its transcription phenotype revealed by whole-transcriptome profiling. *Plant Physiology* **135**: 1069–1083.

Koizumi K, Wu S, MacRae-Crerar A, Gallagher KL. 2011. An essential protein that interacts with endosomes and promotes movement of the SHORT-ROOT transcription factor. *Current Biology* **21**: 1559–1564.

Kurup S, Runions J, Köhler U, Laplaze L, Hodge S, Haseloff J. 2005. Marking cell lineages in living tissues. *Plant Journal* **42**: 444–453.

Kutter C, Schoeb H, Stadler M, Meins F Jr, Si-Ammour A. 2007. MicroRNA-mediated regulation of stomatal development in *Arabidopsis*. *Plant Cell* **19**: 2417–2429.

Lai LB, Nadeau JA, Lucas J, *et al.* 2005. The *Arabidopsis* R2R3 MYB proteins FOUR LIPS and MYB88 restrict divisions late in the stomatal cell lineage. *Plant Cell* **17**: 2754–2767.

Lampard GR, MacAlister CA, Bergmann DC. 2008. *Arabidopsis* stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS. *Science* **322**: 1113–1116.

Laplaze L, Benkova E, Casimiro I, *et al.* 2007. Cytokinins act directly on lateral root founder cells to inhibit root initiation. *Plant Cell* **19**: 3889–3900.

Laskowski M. 2013. Lateral root initiation is a probabilistic event whose frequency is set by fluctuating levels of auxin response. *Journal of Experimental Botany* **64**: 2609–2617.

Laskowski M, Grieneisen VA, Hofhuis H, *et al.* 2008. Root system architecture from coupling cell shape to auxin transport. *PLoS Biology* **6**: 2721–2735.

Lauffenburger DA, Horwitz AF. 1996. Cell migration: a physically integrated molecular process. *Cell* **84**: 359–369.

Laux T, Mayer KFX, Berger J, Jurgens G. 1996. The WUSCHEL gene is required for shoot and floral meristem integrity in *Arabidopsis*. *Development* **122**: 87–96.

Lavenus J, Goh T, Roberts I, *et al.* 2013. Lateral root development in *Arabidopsis*: fifty shades of auxin. *Trends in Plant Science* **18**: 450–458.

Le BH, Cheng C, Bui AQ, *et al.* 2010. Global analysis of gene activity during *Arabidopsis* seed development and identification of seed-specific transcription factors. Proceedings of the National Academy of Sciences of the USA **107**: 8063–8070.

Le J, Liu X-G, Yang K-Z, *et al.* 2014. Auxin transport and activity regulate stomatal patterning and development. *Nature Communications* **5**: 3090.

Le Borgne R, Bardin A, Schweisguth F. 2005. The roles of receptor and ligand endocytosis in regulating Notch signaling. *Development* **132**: 1751–1762.

Lease KA, Lau NY, Schuster RA, Torii KU, Walker JC. 2001. Receptor serine/threonine protein kinases in signalling: analysis of the erecta receptor-like kinase of *Arabidopsis thaliana*. *New Phytologist* **151**: 133–143.

Lee C, Clark SE. 2013. Core pathways controlling shoot meristem maintenance. *Wiley Interdisciplinary Reviews: Developmental Biology* **2**: 671–684.

Lee JS, Kuroha T, Hnilova M, *et al.* 2012. Direct interaction of ligand-receptor pairs specifying stomatal patterning. *Genes & Development* **26**: 126–136.

Levesque MP, Vernoux T, Busch W, *et al*. 2006. Whole-genome analysis of the SHORT-ROOT developmental pathway in *Arabidopsis*. *PLoS Biology* **4**: 739–752.

Li X, Mo X, Shou H, Wu P. 2006. Cytokinin-mediated cell cycling arrest of pericycle founder cells in lateral root initiation of *Arabidopsis*. *Plant and Cell Physiology* **47**: 1112–1123.

Lu J, Guo S, Ebert BL, *et al*. 2008. MicroRNA-mediated control of cell fate in megakaryocyte-erythrocyte progenitors. *Developmental Cell* **14**: 843–853.

Lucas M, Godin C, Jay-Allemand C, Laplaze L. 2008. Auxin fluxes in the root apex co-regulate gravitropism and lateral root initiation. *Journal of Experimental Botany* **59**: 55–66.

Lucas M, Kenobi K, von Wangenheim D, *et al*. 2013*a*. Lateral root morphogenesis is dependent on the mechanical properties of the overlaying tissues. Proceedings of the National Academy of Sciences of the USA **110**: 5229–5234.

Lucas WJ, Groover A, Lichtenberger R, *et al*. 2013*b*. The plant vascular system: evolution, development and functions. *Journal of Integrative Plant Biology* **55**: 294–388.

Ma L, Xin H, Qu L, *et al*. 2011. Transcription profile analysis reveals that zygotic division results in uneven distribution of specific transcripts in apical/basal cells of tobacco. *PLoS One* **6**: e15971.

MacAlister CA, Ohashi-Ito K, Bergmann DC. 2007. Transcription factor control of asymmetric cell divisions that establish the stomatal lineage. *Nature* **445**: 537–540.

Mahonen AP, Bonke M, Kauppinen L, Riikonen M, Benfey PN, Helariutta Y. 2000. A novel two-component hybrid molecule regulates vascular morphogenesis of the *Arabidopsis* root. *Genes & Development* **14**: 2938–2943.

Malamy JE, Benfey PN. 1997. Organization and cell differentiation in lateral roots of *Arabidopsis* thaliana. *Development* **124**: 33–44.

Malamy JE, Ryan KS. 2001. Environmental regulation of lateral root initiation in *Arabidopsis*. *Plant Physiology* **127**: 899–909.

Manzano C, Ramirez-Parra E, Casimiro I, *et al*. 2012. Auxin and epigenetic regulation of SKP2B, an F-box that represses lateral root formation. *Plant Physiology* **160**: 749–762.

Marhavy P, Vanstraelen M, De Rybel B, *et al*. 2013. Auxin reflux between the endodermis and pericycle promotes lateral root initiation. *EMBO Journal* **32**: 149–158.

Mayer KFX, Schoof H, Haecker A, Lenhard M, Jurgens G, Laux T. 1998. Role of WUSCHEL in regulating stem cell fate in the *Arabidopsis* shoot meristem. *Cell* **95**: 805–815.

Mayer U, Torres-Ruiz RA, Berleth T, Misera S, Jürgens G. 1991. Mutations affection body organization in the *Arabidopsis* embryo. *Nature* **353**: 402–407.

Meng L, Buchanan BB, Feldman LJ, Luan S. 2012. CLE-like (CLEL) peptides control the pattern of root growth and lateral root development in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the USA* **109**: 1760–1765.

Miyashima S, Koi S, Hashimoto T, Nakajima K. 2011. Non-cell-autonomous microRNA165 acts in a dose-dependent manner to regulate multiple differentiation status in the *Arabidopsis* root. *Development* **138**: 2303–2313.

Moreno-Risueno MA, Van Norman JM, Moreno A, Zhang JY, Ahnert SE, Benfey PN. 2010. Oscillating gene expression determines competence for periodic *Arabidopsis* root branching. *Science* **329**: 1306–1311.

Mustroph A, Zanetti ME, Jang CJH, *et al*. 2009. Profiling translatomes of discrete cell populations resolves altered cellular priorities during hypoxia in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the USA* 106: 18843–18848.

Nadeau JA, Sack FD. 2002. Control of stomatal distribution on the *Arabidopsis* leaf surface. *Science* **296**: 1697–1700.

Nakajima K, Sena G, Nawy T, Benfey PN. 2001. Intercellular movement of the putative transcription factor SHR in root patterning. *Nature* **413**: 307–311.

Nardmann J, Werr W. 2013. Symplesiomorphies in the WUSCHEL clade suggest that the last common ancestor of seed plants contained at least four independent stem cell niches. *New Phytologist* **199**: 1081–1092.

Nelson T, Langdale JA. 1992. Developmental genetics of C-4 photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**: 25–47.

Nodine MD, Bartel DP. 2012. Maternal and paternal genomes contribute equally to the transcriptome of early plant embryos. *Nature* **482**: 94–7.

Van Norman JM, Xuan W, Beeckman T, Benfey PN. 2013. To branch or not to branch: the role of pre-patterning in lateral root formation. *Development* **140**: 4301–4310.

Ohashi-Ito K, Bergmann DC. 2006. *Arabidopsis* FAMA controls the final proliferation/differentiation switch during stomatal development. *Plant Cell* **18**: 2493–2505.

Ohashi-Ito K, Bergmann DC. 2007. Regulation of the *Arabidopsis* root vascular initial population by LONESOME HIGHWAY. *Development* **134**: 2959–2968.

Ohashi-Ito K, Fukuda H. 2003. HD-Zip III homeobox genes that include a novel member, ZeHB-13 (Zinnia)/ATHB-15 (*Arabidopsis*), are involved in procambium and xylem cell differentiation. *Plant and Cell Physiology* **44**: 1350–1358.

Okumura K, Goh T, Toyokura K, *et al*. 2013. GNOM/FEWER ROOTS is required for the establishment of an auxin response maximum for *Arabidopsis* lateral root initiation. *Plant and Cell Physiology* **54**: 406–417.

Okushima Y, Fukaki H, Onoda M, Theologis A, Tasaka M. 2007. ARF7 and ARF19 regulate lateral root formation via direct activation of LBD/ASL genes in *Arabidopsis*. *Plant Cell* **19**: 118–130.

Palovaara J, Saiga S, Weijers D. 2013. Transcriptomics approaches in the early *Arabidopsis* embryo. *Trends in Plant Science* **18**: 514–521.

Parizot B, Laplaze L, Ricaud L, *et al*. 2008. Diarch symmetry of the vascular bundle in *Arabidopsis* root encompasses the pericycle and is reflected in distich lateral root initiation. *Plant Physiology* **146**: 140–148.

Parizot B, De Rybel B, Beeckman T. 2010. VisuaLRTC: a new view on lateral root initiation by combining specific transcriptome data sets. *Plant Physiology* **153**: 34–40.

Parizot B, Roberts I, Raes J, Beeckman T, De Smet I. 2012. In silico analyses of pericycle cell populations reinforce their relation with associated vasculature in *Arabidopsis*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **367**: 1479–1488.

Péret B, De Rybel B, Casimiro I, *et al*. 2009. *Arabidopsis* lateral root development: an emerging story. *Trends in Plant Science* **14**: 399–408.

Pillitteri LJ, Torii KU. 2012. Mechanisms of stomatal development. *Annual Review of Plant Biology* **63**: 591–614.

Pillitteri LJ, Sloan DB, Bogenschutz NL, Torii KU. 2007. Termination of asymmetric cell division and differentiation of stomata. *Nature* **445**: 501–505.

Pillitteri LJ, Bogenschutz NL, Torii KU. 2008. The bHLH protein, MUTE, controls differentiation of stomata and the hydathode pore in *Arabidopsis*. *Plant and Cell Physiology* **49**: 934–943.

Pillitteri LJ, Peterson KM, Horst RJ, Torii KU. 2011. Molecular profiling of stomatal meristemoids reveals new component of asymmetric cell division and commonalities among stem cell populations in *Arabidopsis*. *Plant Cell* **23**: 3260–3275.

Rademacher EH, Moller B, Lokerse AS, Llavata-Peris CI, van den Berg W, Weijers D. 2011. A cellular expression map of the *Arabidopsis* AUXIN RESPONSE FACTOR gene family. *Plant Journal* **68**: 597–606.

Rademacher EH, Lokerse AS, Schlereth A, *et al*. 2012. Different auxin response machineries control distinct cell fates in the early plant embryo. *Developmental Cell* **22**: 211–222.

Ragni L, Nieminen K, Pacheco-Villalobos D, Sibout R, Schwechheimer C, Hardtke CS. 2011. Mobile gibberellin directly stimulates *Arabidopsis* hypocotyl xylem expansion. *Plant Cell* **23**: 1322–1336.

Rasmussen CG, Humphries JA, Smith LG. 2011. Determination of symmetric and asymmetric division planes in plant cells. *Annual Review of Plant Biology* **62**: 387–409.

Reinhardt D, Frenz M, Mandel T, Kuhlemeier C. 2003. Microsurgical and laser ablation analysis of interactions between the zones and layers of the tomato shoot apical meristem. *Development* **130**: 4073–4083.

Reinhardt D, Frenz M, Mandel T, Kuhlemeier C. 2005. Microsurgical and laser ablation analysis of leaf positioning and dorsoventral patterning in tomato. *Development* **132**: 15–26.

Ren H, Santner A, del Pozo JC, Murray JAH, Estelle M. 2008. Degradation of the cyclin-dependent kinase inhibitor KRP1 is regulated by two different ubiquitin E3 ligases. *Plant Journal* **53**: 705–716.

Richter GL, Monshausen GB, Krol A, Gilroy S. 2009. Mechanical stimuli modulate lateral root organogenesis. *Plant Physiology* **151**: 1855–1866.

Robinson S, de Reuille PB, Chan J, Bergmann D, Prusinkiewicz P, Coen E. 2011. Generation of spatial patterns through cell polarity switching. *Science* **333**: 1436–1440.

Robischon M, Du J, Miura E, Groover A. 2011. The *Populus* class III HD ZIP, popREVOLUTA, influences cambium initiation and patterning of woody stems. *Plant Physiology* **155**: 1214–1225.

Rogg LE, Lasswell J, Bartel B. 2001. A gain-of-function mutation in IAA28 suppresses lateral root development. *Plant Cell* **13**: 465–480.

Rojo E, Sharma VK, Kovaleva V, Raikhel NV, Fletcher JC. 2002. CLV3 is localized to the extracellular space, where it activates the *Arabidopsis* CLAVATA stem cell signaling pathway. *Plant Cell* **14**: 969–977.

Rudall PJ, Hilton J, Bateman RM. 2013. Several developmental and morphogenetic factors govern the evolution of stomatal patterning in land plants. *New Phytologist* **200**: 598–614.

Ruiz-Herrero T, Estrada J, Guantes R, Miguez DG. 2013. A tunable coarse-grained model for ligand-receptor interaction. *PLoS Computational Biology* **9**: e1003274.

De Rybel B, Vassileva V, Parizot B, *et al*. 2010. A novel Aux/IAA28 signaling cascade activates GATA23-dependent specification of lateral root founder cell identity. *Current Biology* **20**: 1697–1706.

De Rybel B, Audenaert D, Xuan W, *et al*. 2012. A role for the root cap in root branching revealed by the non-auxin probe naxillin. *Nature Chemical Biology* **8**: 798–805.

De Rybel B, Moller B, Yoshida S, *et al*. 2013. A bHLH complex controls embryonic vascular tissue establishment and indeterminate growth in arabidopsis. *Developmental Cell* **24**: 426–437.

Sabatini S, Heidstra R, Wildwater M, Scheres B. 2003. SCARECROW is involved in positioning the stem cell niche in the *Arabidopsis* root meristem. *Genes & Development* **17**: 354–358.

Sankar M, Nieminen K, Ragni L, Xenarios I, Hardtke CS, Traas J. 2014. Automated quantitative histology reveals vascular morphodynamics during *Arabidopsis* hypocotyl secondary growth. *eLife* **3**.

Sanz L, Dewitte W, Forzani C, *et al*. 2011. The *Arabidopsis* D-type cyclin CYCD2;1 and the inhibitor ICK2/KRP2 modulate auxin-induced lateral root formation. *Plant Cell* **23**: 641–660.

Sarkar AK, Luijten M, Miyashima S, *et al*. 2007. Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. *Nature* **446**: 811–814.

Savage NS, Walker T, Wieckowski Y, Schiefelbein J, Dolan L, Monk NAM. 2008. A mutual support mechanism through intercellular movement of CAPRICE and GLABRA3 can pattern the *Arabidopsis* root epidermis. *PLoS Biology* **6**: 1899–1909.

Scheres B. 2007. Stem-cell niches: nursery rhymes across kingdoms. *Nature Reviews. Molecular Cell Biology* **8**: 345–354.

Scheres B, Wolkenfelt H, Willemsen V, *et al*. 1994. Embryonic origin of the *Arabidopsis* primary root and root meristem initials. *Development* **120**: 2475–2487.

Scheres B, Dilaurenzio L, Willemsen V, *et al*. 1995. Mutations affecting the radial organization of the *Arabidopsis* root display specific defects throughout the embryonic axiS. *Development* **121**: 53–62.

Schlereth A, Moller B, Liu W, *et al*. 2010. MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. *Nature* **464**: 913–916.

Schoof H, Lenhard M, Haecker A, Mayer KFX, Jurgens G, Laux T. 2000. The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell* **100**: 635–644.

Schrader J, Baba K, May ST, *et al*. 2003. Polar auxin transport in the wood-forming tissues of hybrid aspen is under simultaneous control of developmental and environmental signals. Proceedings of the National Academy of Sciences of the USA **100**: 10096–10101.

Schrader J, Nilsson J, Mellerowicz E, *et al*. 2004. A high-resolution transcript profile across the wood-forming meristem of poplar identifies potential regulators of cambial stem cell identity. *Plant Cell* **16**: 2278–2292.

Schweisguth F. 2004. Regulation of notch signaling activity. *Current Biology* **14**: R129–R138.

Shpak ED, McAbee JM, Pillitteri LJ, Torii KU. 2005. Stomatal patterning and differentiation by synergistic interactions of receptor kinases. *Science* **309**: 290–293.

De Smet I. 2012. Lateral root initiation: one step at a time. *New Phytologist* **193**: 867–873.

De Smet I, Beeckman T. 2011. Asymmetric cell division in land plants and algae: the driving force for differentiation. *Natutr Reviews. Molecular Cell Biology* **12**: 177–188.

De Smet I, Tetsumura T, De Rybel B, *et al*. 2007. Auxin-dependent regulation of lateral root positioning in the basal meristem of *Arabidopsis*. *Development* **134**: 681–690.

De Smet I, Vassileva V, De Rybel B, *et al*. 2008. Receptor-like kinase ACR4 restricts formative cell divisions in the *Arabidopsis* root. *Science* **322**: 594–597.

De Smet I, Lau S, Mayer U, Jurgens G. 2010*a*. Embryogenesis – the humble beginnings of plant life. *Plant Journal* **61**: 959–970.

De Smet I, Lau S, Voβ U, *et al*. 2010*b*. Bimodular auxin response controls organogenesis in *Arabidopsis*. Proceedings of the National Academy of Sciences of the USA **107**: 2705–2710.

Smith S, De Smet I. 2012. Root system architecture: insights from *Arabidopsis* and cereal crops. *Philosophical Transactions of the Royal Society B: Biological Sciences* **367**: 1441–1452.

Song SK, Lee MM, Clark SE. 2006. POL and PLL1 phosphatases are CLAVATA1 signaling intermediates required for *Arabidopsis* shoot and floral stem cells. *Development* **133**: 4691–4698.

Sozzani R, Iyer-Pascuzzi A. 2014. Postembryonic control of root meristem growth and development. *Current Opinion in Plant Biology* **17**: 7–12.

Sozzani R, Cui H, Moreno-Risueno MA, *et al*. 2010. Spatiotemporal regulation of cell-cycle genes by SHORTROOT links patterning and growth. *Nature* **466**: 128–132.

Spana EP, Doe CQ. 1995. The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*. *Development* **121**: 3187–3195.

Spencer MW, Casson SA, Lindsey K. 2007. Transcriptional profiling of the *Arabidopsis* embryo. *Plant Physiology* **143**: 924–940.

Suer S, Agusti J, Sanchez P, Schwarz M, Greb T. 2011. WOX4 imparts auxin responsiveness to cambium cells in *Arabidopsis*. *Plant Cell* **23**: 3247–3259.

Swarup K, Benkova E, Swarup R, *et al*. 2008. The auxin influx carrier LAX3 promotes lateral root emergence. *Nature Cell Biology* **10**: 946–954.

Tanaka H, Watanabe M, Watanabe D, Tanaka T, Machida C, Machida Y. 2002. ACR4, a putative receptor kinase gene of *Arabidopsis thaliana*, that is expressed in the outer cell layers of embryos and plants, is involved in proper embryogenesis. *Plant and Cell Physiology* **43**: 419–428.

Toyota M, Gilroy S. 2013. Gravitropism and mechanical signaling in plants. *American Journal of Botany* **100**: 111–125.

Truernit E, Bauby H, Dubreucq B, *et al*. 2008. High-resolution whole-mount imaging of three-dimensional tissue organization and gene expression enables the study of phloem development and structure in *Arabidopsis*. *Plant Cell* **20**: 1494–1503.

Truernit E, Baub H, Belcram K, Barthelemy J, Palauqui J-C. 2012. OCTOPUS, a polarly localised membrane-associated protein, regulates phloem differentiation entry in *Arabidopsis thaliana*. *Development* **139**: 1306–1315.

Uchida N, Tasaka M. 2013. Regulation of plant vascular stem cells by endodermis-derived EPFL-family peptide hormones and phloem-expressed ERECTA-family receptor kinases. *Journal of Experimental Botany* **64**: 5335–5343.

Ueda M, Zhang Z, Laux T. 2011. Transcriptional activation of *Arabidopsis* axis patterning genes WOX8/9 links zygote polarity to embryo development. *Developmental Cell* **20**: 264–270.

Vanneste S, De Rybel B, Beemster GTS, *et al*. 2005. Cell cycle progression in the pericycle is not sufficient for SOLITARY ROOT/IAA14-mediated lateral root initiation in *Arabidopsis thaliana*. *Plant Cell* **17**: 3035–3050.

Vanneste S, Coppens F, Lee E, *et al*. 2011. Developmental regulation of CYCA2 s contributes to tissue-specific proliferation in *Arabidopsis*. *EMBO Journal* **30**: 3430–3441.

Vanstraelen M, Beeckman T. 2013. Traffic control in the root: keeping root branching in check. *Developmental Cell* **26**: 113–114.

Vermeer JEM, von Wangenheim D, Barberon M, *et al*. 2014. a spatial accommodation by neighboring cells is required for organ initiation in *Arabidopsis*. *Science* **343**: 178–183.

Wang J, Kucukoglu M, Zhang L, *et al*. 2013. The *Arabidopsis* LRR-RLK, PXC1, is a regulator of secondary wall formation correlated with the TDIF-PXY/TDR-WOX4 signaling pathway. *BMC Plant Biology* **13**: 94.

Weigel D, Jurgens G. 2002. Stem cells that make stems. *Nature* **415**: 751–754.

Weijers D, Schlereth A, Ehrismann JS, Schwank G, Kientz M, Jurgens G. 2006. Auxin triggers transient local signaling for cell specification in *Arabidopsis* embryogenesis. *Developmental Cell* **10**: 265–270.

Welch D, Hassan H, Blilou I, Immink R, Heidstra R, Scheres B. 2007. *Arabidopsis* JACKDAW and MAGPIE zinc finger proteins delimit asymmetric cell division and stabilize tissue boundaries by restricting SHORT-ROOT action. *Genes & Development* **21**: 2196–2204.

Wenzel CL, Rost TL. 2001. Cell division patterns of the protoderm and root cap in the 'closed' root apical meristem of *Arabidopsis thaliana*. *Protoplasma* **218**: 203–213.

Wenzel CL, Hester Q, Mattsson J. 2008. Identification of genes expressed in vascular tissues using NPA-induced vascular overgrowth in *Arabidopsis*. *Plant and Cell Physiology* **49**: 457–468.

Whitford R, Fernandez A, De Groodt R, Ortega E, Hilson P. 2008. Plant CLE peptides from two distinct functional classes synergistically induce division of vascular cells. *Proceedings of the National Academy of Sciences of the USA* **105**: 18625–18630.

Wilmoth JC, Wang S, Tiwari SB, *et al*. 2005. NPH4/ARF7 and ARF19 promote leaf expansion and auxin-induced lateral root formation. *Plant Journal* **43**: 118–130.

Xiang D, Venglat P, Tibiche C, *et al*. 2011. Genome-wide analysis reveals gene expression and metabolic network dynamics during embryo development in *Arabidopsis*. *Plant Physiology* **156**: 346–356.

Yadav SR, Bishopp A, Helariutta Y. 2010. Plant development: early events in lateral root initiation. *Current Biology* **20**: R843–R845.

Yordanov YS, Regan S, Busov V. 2010. Members of the LATERAL ORGAN BOUNDARIES DOMAIN transcription factor family are involved in the regulation of secondary growth in *Populus*. *Plant Cell* **22**: 3662–3677.

Yu LP, Miller AK, Clark SE. 2003. POLTERGEIST encodes a protein phosphatase 2C that regulates CLAVATA pathways controlling stem cell identity at *Arabidopsis* shoot and flower meristems. *Current Biology* **13**: 179–188.

Zhang HM, Forde BG. 1998. An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. *Science* **279**: 407–409.

Zhang J, Nieminen K, Serra JAA, Helariutta Y. 2014. The formation of wood and its control. *Current Opinion in Plant Biology* **17**: 56–63.

Zhang X, Facette M, Humphries JA, *et al*. 2012. Identification of PAN2 by quantitative proteomics as a leucine-rich repeat-receptor-like kinase acting upstream of PAN1 to polarize cell division in maize. *Plant Cell* **24**: 4577–4589.

Zhang Z, Laux T. 2011. The asymmetric division of the *Arabidopsis* zygote: from cell polarity to an embryo axis. *Sexual Plant Reproduction* **24**: 161–169.

Zhao C, Sun G, Li S, Shi Y. 2009. A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. *Nature Structural & Molecular Biology* **16**: 365–371.

Zhu Y, Song D, Sun J, Wang X, Li L. 2013. PtrHB7, a class III HD-zip gene, plays a critical role in regulation of vascular cambium differentiation in *Populus*. *Molecular Plant* **6**: 1331–1343.