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INVITED REVIEW

Genetic control of root growth: from genes to networks

Radka Slovak, Takehiko Ogura, Santosh B. Satbhai, Daniela Ristova and Wolfgang Busch*

Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna Biocenter (VBC), Dr. Bohr-Gasse 3, 1030 Vienna, Austria
** For correspondence. E-mail wolfgang.busch@gmi.oeaw.ac.at*

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• **Background** Roots are essential organs for higher plants. They provide the plant with nutrients and water, anchor the plant in the soil, and can serve as energy storage organs. One remarkable feature of roots is that they are able to adjust their growth to changing environments. This adjustment is possible through mechanisms that modulate a diverse set of root traits such as growth rate, diameter, growth direction and lateral root formation. The basis of these traits and their modulation are at the cellular level, where a multitude of genes and gene networks precisely regulate development in time and space and tune it to environmental conditions.
• **Scope** This review first describes the root system and then presents fundamental work that has shed light on the basic regulatory principles of root growth and development. It then considers emerging complexities and how they have been addressed using systems-biology approaches, and then describes and argues for a systems-genetics approach. For reasons of simplicity and conciseness, this review is mostly limited to work from the model plant *Arabidopsis thaliana*, in which much of the research in root growth regulation at the molecular level has been conducted.
• **Conclusions** While forward genetic approaches have identified key regulators and genetic pathways, systems-biology approaches have been successful in shedding light on complex biological processes, for instance molecular mechanisms involving the quantitative interaction of several molecular components, or the interaction of large numbers of genes. However, there are significant limitations in many of these methods for capturing dynamic processes, as well as relating these processes to genotypic and phenotypic variation. The emerging field of systems genetics promises to overcome some of these limitations by linking genotypes to complex phenotypic and molecular data using approaches from different fields, such as genetics, genomics, systems biology and phenomics.

Key words: Root, root development, root growth, genetics, root patterning, systems biology, modelling, systems genetics, networks, *Arabidopsis thaliana*.

INTRODUCTION

Mostly hidden from view, below the ground, roots constitute an essential organ for higher plants. They provide the plant with nutrients and water, anchor the plant in the soil, and can serve as energy storage organs. The ability of roots to acquire minerals and water from the soil determines, to a large extent, the ability of a plant to grow (Zobel, 1986). The root system is frequently exposed to a multitude of environmental constraints (such as drought, extreme temperature, lack of essential nutrients, exposure to toxic minerals, and soil compaction) and it is one remarkable feature of roots that they are able to adjust their growth to such changing environments (Malamy, 2005). This adjustment is realized by the tuning of a diverse set of root traits such as growth rate, diameter, growth direction and lateral root (LR) formation. These traits shape the root system architecture (RSA), which is the spatial configuration of roots in the soil. The RSA shows a significant degree of plasticity in response to the heterogeneous distribution of soil resources and variations of soil conditions (Lynch, 1995*a*). Understanding which regulatory processes and underlying genetic components regulate root growth, and thereby RSA, is not only a fascinating question of basic biology but also key to breed and engineer better performing plants. However, it has become clear that root growth regulation is a highly complicated process and is controlled at many different levels by complex actions of gene networks in both time and space. In this review we will describe fundamental work that has shed light on the basic principles in root growth and development, will proceed with the emerging complexities and how they have initially been addressed, and finally describe and argue for a systems-genetics approach. Throughout this review, due to reasons of simplicity and extent, we will mostly limit ourselves to the work in the model plant arabidopsis (*Arabidopsis thaliana*), for which much of the research in root growth regulation at the molecular level has been conducted.

THE ROOT SYSTEM

The distribution and spatial configuration of the roots in the soil – the RSA – is a fundamentally important physiological parameter for plants. It is determined by the shapes, sizes and three-dimensional distribution of roots, as well as by the branching arrangement of the primary and higher order roots. Other important factors are the root hair density and length; increases in length and/or number of root hairs can dramatically increase the root–soil interface. Essentially, RSA determines the volume

of soil that is explored by the roots (given by the depth and breadth of the extent of the root system) and the root surface area that interfaces with the soil. The volume of soil that is explored determines the zone from which mobile nutrients can be acquired, and the root surface area determines the zone from which immobile nutrients can be acquired (Bray, 1954). RSA varies between species and also displays significant natural variation within a species (Hochholdinger and Tuberosa, 2009; Giehl *et al*., 2012; Gruber *et al*., 2013; Rosas *et al*., 2013).

In dicots such as arabidopsis, the root system consists of a single primary root (PR) of embryonic origin, which often remains active throughout the plant's life cycle and can develop several orders of LRs (Osmont *et al*., 2007), and post-embryonically derived junction roots formed at the collet, the junction between the hypocotyl and root (Falasca and Altamura, 2003). In monocots such as maize (*Zea mays*) and rice (*Oryza sativa*), the root system contains embryonic primary and seminal roots (SRs), and post-embryonic shoot-borne roots and LRs (Hochholdinger and Tuberosa, 2009). The PRs are the first root to emerge in both dicots and monocots, and are derived from embryonically formed meristematic tissue at the root tip called the root apical meristem (RAM). The RAM consists of a basal stem cell pool around an organizing centre called the quiescent centre (QC; Dolan *et al*., 1993). Root hairs are formed in the differentiation zone of the root from specialized epidermal cells called trichoblasts, and vastly increase the root surface area and contribute to the acquisition of immobile nutrients (Bates and Lynch, 1996). LRs are the most important root class for the RSA. In arabidopsis and other dicots, LRs are derived from pericycle cells adjacent to the xylem tissue (xylem-pole pericycle) (Dubrovsky *et al*., 2000). In monocots such as maize and rice, they are initiated in the phloem-pole pericycle, and cells derived from the pericycle and endodermis contribute to the LRs (De Smet *et al*., 2006).

THE GENETIC BASIS OF ROOT GROWTH AND DEVELOPMENT

The root system and RSA are the results of continuous root growth and development. Our understanding of root growth regulation and development, and their consequence, RSA, is most advanced in the model plant arabidopsis. The available genome sequence (Arabidopsis Genome Initiative, 2000) together with the short generation time made it perfectly suited for genetic approaches. Moreover, the ease of genetic and molecular manipulation and the steadily growing number of genetic, biochemical and computational materials in the arabidopsis research community have resulted in making arabidopsis the most thoroughly investigated plant species. Root research in this model has profited from the root's optical transparency, its small diameter and simplicity of organization, the rigid order of cell divisions and unusually invariant cellular lineages, and, finally, the ability to grow it easily in large numbers on sterile agar plates. Consequently, the arabidopsis root has been used to address not only questions related to root physiology but also basic questions in developmental and cell biology. In this section, we review genetic and molecular processes underlying root development, and then highlight how these mechanisms impact root growth. We note that in this section, we mainly restrict ourselves to highlight seminal work identifying key genes and mechanisms with classical approaches and will review more recent work that used systems-biology approaches for the same purpose in a later section.

Cell fate specification in the root

The function of the root is highly dependent on its cellular architecture, with precisely defined cell types radially arranged in cell files around the central axis of the root. A key question for root biology was therefore how this precise patterning is regulated. Landmark studies conducted clonal analyses to reveal the origin of the cell files, identifying initial cells that give rise to specific cell lineages (Dolan *et al*., 1994; Scheres *et al*., 1994). While the regularity of cell divisions (Dolan *et al*., 1993; Baum and Rost, 1996) and the rigid cellular organization were suggestive of lineage-based determinants strictly controlling cell fate (van den Berg *et al*., 1995; Kidner *et al*., 2000), laser ablation studies showed that positional cues are critical for cell fate determination (van den Berg *et al*., 1995). In particular, the ablation of specific cells enabled neighbouring cells from another cell file to occupy the freed space and adopt the developmental fate of the removed cells, as revealed by the expression of tissue-specific marker genes (van den Berg *et al*., 1995). These studies highlighted one of many examples of the developmental plasticity of root cells.

Ground tissue. Cell fate specification is largely governed by the activity of key transcription factors. For instance, roots of the GRAS family transcription factor mutants *shortroot* (*shr*) and *scarecrow* (*scr*) contain only one ground tissue layer that usually consists of the endodermis and cortex cell layers (Benfey *et al*., 1993; Scheres *et al*., 1995). While the *shortroot* ground tissue layer is entirely lacking endodermal differentiation markers (Benfey *et al*., 1993), the ground tissue in *scarecrow* has features of both cortex and endodermis (Scheres *et al*., 1995; Di Laurenzio *et al*., 1996). Subsequent studies indicated that *SHR* as well as *SCR* are both necessary for the periclinal cell division of the daughter cell of the cortex endodermis initial (CEI) that gives rise to both cell types of ground tissue initial daughter cells (Scheres *et al*., 1995). However, only *SHR* is required for the specification of endodermal cell fate (Benfey *et al*., 1993; Helariutta *et al*., 2000; Nakajima *et al*., 2001). Intriguingly, *SHR* mRNA is not expressed in the ground tissue but only in the stele (pericycle and vascular tissues). This non-cell-autonomous function of SHR is due to the movement of the SHR protein from the stele to the ground tissue (Nakajima *et al*., 2001). There, SHR directly induces the expression of *SCR* (Levesque *et al*., 2006). In turn, SCR sequesters SHR to the endodermal cell nucleus, thereby preventing SHR movement (Fig. 1) and further upregulating *SCR* expression, thus giving rise to a positive feedback loop that ensures the appropriate timing and location of the cell division (Cui *et al*., 2007). Further studies, using tools of systems biology, identified additional key genes involved in the *SHR*/*SCR* regulatory circuit and eventually led to a mathematical model of this patterning process (see later). Apart from these efforts, other ground tissue patterning genes have been identified. For instance, *SCHIZORHIZA* (*SCZ*), a gene that belongs to the heat shock factor family of transcription factors, is required for the

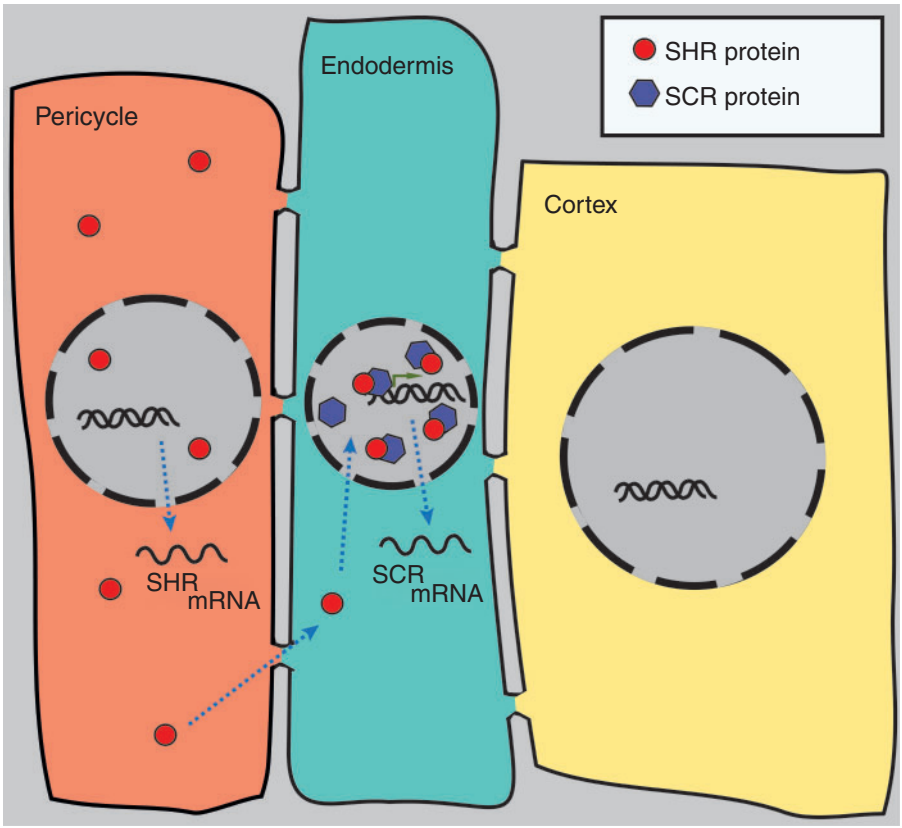

FIG. 1. Model for SHR/SCR activity in ground tissue patterning. SHR mRNA and protein are expressed in the pericycle. SHR protein moves to the endodermis, where it increases *SCR* expression and is in turn sequestered by SCR to the nucleus. This constitutes a positive feedback loop, reinforcing the inhibition of further cell–cell movement of SHR.

establishment of ground tissue stem cells in the embryonic root (Pernas *et al*., 2010). The *scz* mutant develops supernumerary layers of ground tissue and root hairs originating from the subepidermal layer (Mylona *et al*., 2002), implying a role for *SCZ* in suppressing both extranumerary periclinal cell divisions and epidermal cell fate in CEI daughter cells (Mylona *et al*., 2002).

Epidermis. Cell fate specification of cells within one tissue has been studied intensely in the epidermis – a root tissue consisting of two cell types, hair-forming trichoblasts and hairless atrichoblasts. The hair cell files are located adjacent to the anticlinal cell wall between two cortical cell files (Fig. 2). Each hairless cell file is located above a single cortical cell file, thus not spanning anticlinal cell boundaries. The epidermal cell pattern is already established during embryogenesis (Costa and Dolan, 2003). Both cell types are distinguishable as trichoblasts and atrichoblasts (Fig. 3A) long before the emergence of root hairs (Dolan *et al*., 1994; Galway *et al*., 1994; Berger *et al*., 1998*a*). Laser ablation experiments showed that cell fate determination of hair-bearing and hairless cells is dependent on position-dependent cell–cell communication, rather than lineage-related determinants (Berger *et al*., 1998*b*). Surgical experiments in radish (Bünning, 1951), another member of the *Brassicaceae* family, pointed to the hair cell fate being the default fate of epidermal cells.

Genetic screens provided insight into the specification of hair vs. hairless cells, and numerous mutants with impaired root epidermal cell fate determination have been discovered. These include genes required for hairless cell fate such as *WEREWOLF* (*WER*), *TRANSPARENT TESTA GLABRA* (*TTG1*) and *GLABRA2* (*GL2*) (Galway *et al*., 1994; Rerie *et al*., 1994; Masucci *et al*., 1996; Lee and Schiefelbein, 1999) and the redundantly acting *GLABRA3* (*GL3*) and *ENHANCER OF GLABRA 3* (*EGL3*) (Bernhardt *et al*., 2003). Single loss-of-function mutants of the last two genes show only slight increases in root hair production, while *gl3 egl3* double mutants form ectopic hair cells (Bernhardt *et al*., 2003). In contrast to hairless cell fate-specifying genes, *CAPRICE* (*CPC*) promotes hair cell fate specification, *cpc* having irregularly distributed and dramatically reduced numbers of root hairs (Wada *et al*., 1997).

In atrichoblast cells, the hairless cell fate-promoting genes (*WER*, *GL3*, *EGL3* and *TTG1*) act upstream of *GL2* and *CPC* (Hung *et al*., 1998; Lee and Schiefelbein, 1999; Bernhardt *et al*., 2003). Both GL3 and EGL3 directly interact with WER (Bernhardt *et al*., 2003) and TTG1 in yeast two-hybrid (Y2H) assays (Payne *et al*., 2000; Esch *et al*., 2003; Zhang *et al*., 2003). This evidence of protein–protein interaction suggested an activator complex consisting of WER, GL3, EGL3 and TTG1 (Zhang *et al*., 2003). This complex binds to the promoter regions of *GL2* and *CPC*, and directly activates their transcription (Koshino-Kimura *et al*., 2005). While *GL2* specifies hairless cell fate (Masucci *et al*., 1996) and therefore acts cell autonomously, *CPC* is required for hair cell fate determination (Wada *et al*., 1997) and acts non-autonomously (Wada *et al*.,

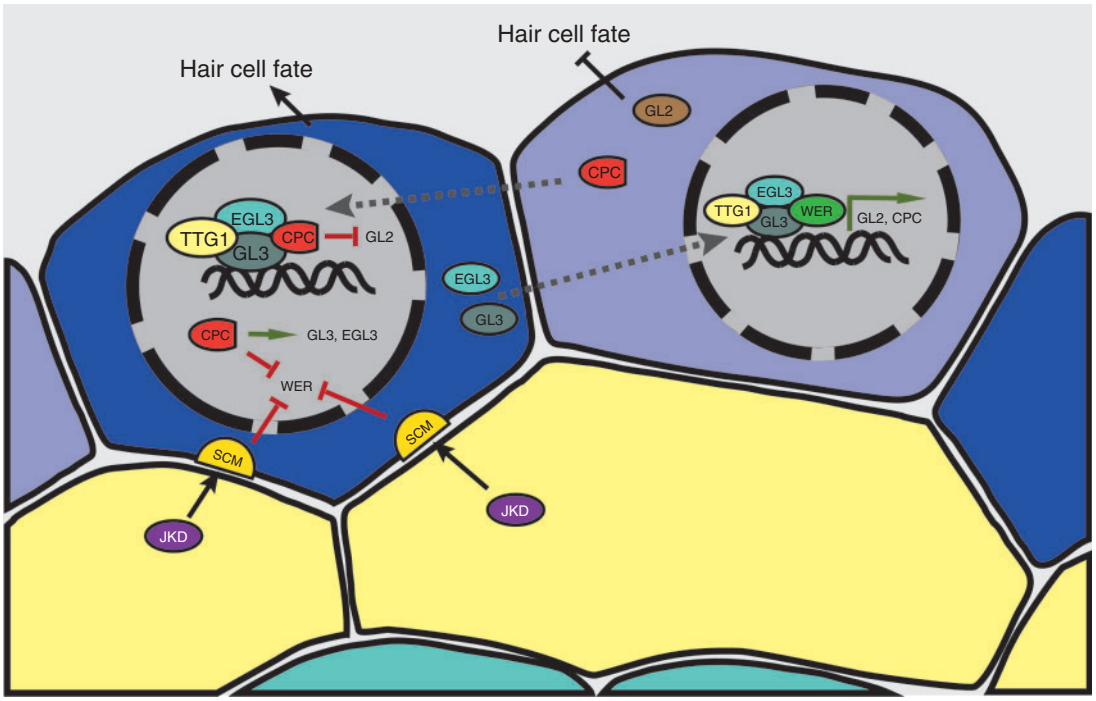

FIG. 2. Model for epidermis cell fate specification. The TTG1/GL3/EGL3/WER complex directly activates expression of *GL2* and *CPC* in atrichoblast cells (in light blue). CPC moves circumferentially to neighbouring cells, there outcompetes WER in binding to TTG1/GL3/EGL3 and results in the downregulation of *GL2* and promotion of hair cell identity (trichoblast in dark blue). JKD and SCM provide positional cues from the underlying cortex cell layer by repressing *WER* and tipping the balance in favour of trichoblast cell fate.

2002) by moving circumferentially to neighbouring cells (Wada *et al*., 2002; Kurata *et al*., 2005). Since CPC interacts with both GL3 and EGL3 in yeast cells (Bernhardt *et al*., 2003), a competition model between the R2R3-type MYB transcription factor WER and the R3-type MYB transcription factor CPC was suggested (Lee and Schiefelbein, 1999) and later on experimentally confirmed (Bernhardt *et al*., 2005; Tominaga *et al*., 2007). While TTG1/GL3/EGL3 interact in hairless cells with WER, interaction with the mobile CPC protein that accumulates in hair cells generates an inactive complex (Bernhardt *et al*., 2005). This leads to the downregulation of *GL2* and results in hair cell fate. Moreover accumulation of CPC leads to downregulation of *WER* and upregulation of *GL3* and *EGL3* (Bernhardt *et al*., 2005), respectively. In turn, GL3 and EGL3 move from root hair cells to neighbouring hairless cells and facilitate the assembly of the active transcription complex TTG1/GL3/EGL3/WER which leads to *GL2* upregulation and reinforces the hairless cell fate (Bernhardt *et al*., 2005). Overall, this complex regulation constitutes a bilateral inhibition feedback loop that leads to a high stability of epidermal patterning (Savage *et al*., 2008). Two genes are key factors for providing the initial positional information for the epidermal cell patterning from the underlying cortex cell layer. These genes encode a receptor-like kinase SCRAMBLED (SCM; Kwak and Schiefelbein, 2008; Schiefelbein *et al*., 2009) and a zinc finger protein JACKDAW (JKD; Hassan *et al*., 2010). *SCM* is epistatic to *JKD*, and both single mutants show ectopic expression of WER, GL2 and CPC proteins (Hassan *et al*., 2010). Furthermore, systems-biology approaches have identified further components for epidermal patterning (see later).

Stele. Of all tissues in the root, the stele, a composite tissue that consists of several cell types and contains the vascular bundles, displays the highest degree of complexity during patterning and differentiation. This enables drastic terminal differentiation events that lead to highly specialized vasculature cells, such as dead vessel elements or sieve elements lacking a nucleus. Many important molecular pathways and regulators that are involved in stele patterning and differentiation of the vasculature have been identified. These involve a variety of factors and pathways such as hormones, transcription factors and microRNAs (miRNAs; a very comprehensive summary of the current state in the field is given in Lucas *et al*., 2013). Here we highlight several key patterning events in the stele.

Different cell types in the stele are generated through a set of asymmetric cell divisions. *wooden leg 1*/*cytokinin response 1* (*wol*/*cre1*) mutants contain a stele that has a reduced number of cell files (Scheres *et al*., 1995) since these plants are impaired in executing a number of periclinal asymmetric cell divisions. This results in the specification of all vascular cells in *wol*/*cre1* mutants as protoxylem cells (Mähönen *et al*., 2000). The reduced number of vascular cylinder cells can be phenocopied by decreasing cytokinin levels specifically in the procambium using a tissue-specifically expressed version of the *CYTOKININ OXIDASE2*-encoded enzyme (Mähönen *et al*., 2006). Consistent with that, a suppressor screen for the *wol*/*cre1* phenotype identified *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*), a negative regulator of cytokinin signalling, supporting the key role of cytokinin signalling in phloem and metaxylem vascular tissue morphogenesis (Mähönen *et al*., 2006). Interestingly, non-cell-autonomous

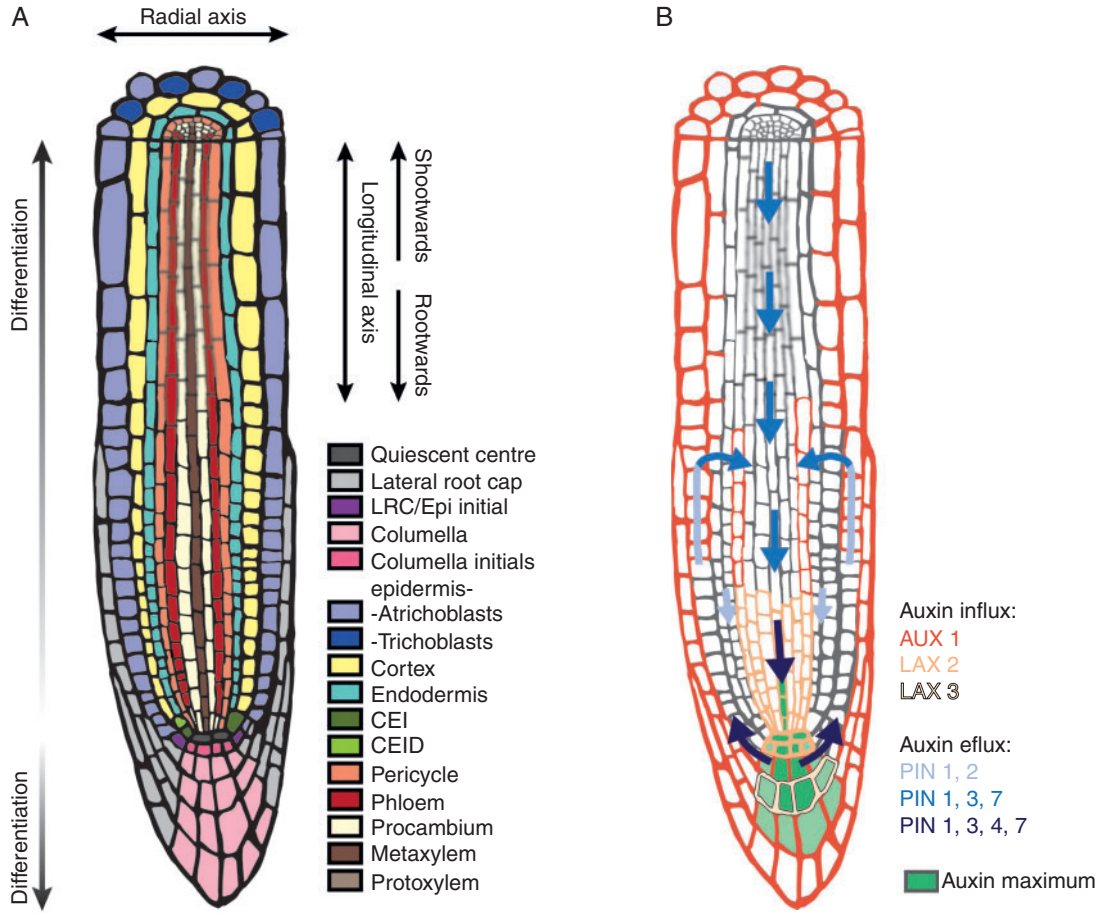

FIG. 3. Root apical–basal patterning. (A) Schematic of the root apical patterning. (B) A simplified auxin reflux loop in the root apex. The direction of the auxin flow is mediated by PIN efflux carriers.

movement of small RNAs also conveys positional information and contributes to the patterning of the stele. In particular, the *SHR*/*SCR* module induces the expression of two miRNA genes *MIR165a* and *MIR166b* in the endodermis. MicroRNAs 165 and 166 subsequently move to the stele. There, they downregulate the expression of the class III HD-ZIP transcription factor *PHABULOSA*. This activity is necessary for xylem (Carlsbecker *et al*., 2010), pericycle and ground tissue patterning (Miyashima *et al*., 2011). *phb* mutants, expressing miRNA-resistant *PHB* transcripts with insertion in the miR165/166 target site, contain a stele with fewer cell files and rare extranumerary cortex cell files (Miyashima *et al*., 2011), demonstrating the critical role of miRNAs in cell fate determination. Moreover, the centripetal movement of miRNAs 165 and 166 results in differential distribution of their target PHB and subsequent dosage-dependent specification of the xylem cell types. Low levels of PHB determine protoxylem, and higher levels of PHB determine metaxylem cell fate (Carlsbecker *et al*., 2010). Another critical gene for controlling the number of cell files in the vascular bundle is *LONESOME HIGHWAY*. It does not appear to be necessary for any particular cell type determination, but for the bilateral symmetry of the root vasculature. *lhw* roots are formed with only one xylem and phloem pole (Ohashi-Ito and Bergmann, 2007). LHW forms hetordimers with another basic helix–loop–helix (bHLH) protein, TARGET OF MONOPTEROS5 (TMO5) (De Rybel *et al*., 2013), and both are critical for the periclinal cell divisions necessary for the establishment of correct patterning of the vascular bundle. Light was shed on phloem cell fate specification by characterization of the *altered phloem development* (*apl*) mutant. *apl* seedlings exhibit finite root growth as well as arrested shoot development. Ultimately this mutation is lethal for seedlings. Detailed analysis of the defects in *apl* mutants revealed impaired asymmetric cell divisions. Phloem cell differentiation is also disrupted in *apl* mutants, with cells in the position of the phloem adopting xylem features. This implies a role for APL in promoting phloem cell identity determination as well as repressing xylem specification at phloem poles in the root vasculature (Bonke *et al*., 2003).

Root cap. The root cap constitutes a protective layer of cells in front of the RAM. A remarkable feature of this tissue is that root cap cells, during their differentiation, are continuously removed from the root tip. The removal of root cap cells is a combination of shedding of living cells at the root tip and programmed cell death (PCD) in the shootward part of the LR cap (Fendrych *et al*., 2014). Cells of the root cap originate from the root cap initial cells. Key genes for root cap cell fate determination are the NAC domain transcription factor genes *FEZ* and

SOMBRERO (*SMB*; Willemsen *et al*., 2008). These genes control the timing and orientation of divisions of the root cap initials. *FEZ* is expressed in the epidermis/LR cap initial cell and columella initial cell prior to its periclinal cell division. FEZ activates expression of *SMB* in the daughter cells of the root cap initials. SMB in turn mediates the repression of *FEZ*, thereby closing a feedback loop and thus suppressing further periclinal divisions in the root cap daughter cells (Willemsen *et al*., 2008). Together with two other NAC transcription factor genes, *BEARSKIN1* (*BRN1*) and *BEARSKIN2* (*BRN2*), *SMB* controls the maturation of the root cap (Bennett *et al*., 2010). Lateral root cap cells are not cleared off in the *smb* mutant line due to the critical role of SMB in PCD of these cells (Fendrych *et al*., 2014), while in the *brn1 brn2* double mutant columella cells do not detach and continue to divide. In the *smb brn1 brn2* triple mutant, all root cap cells fail to mature (Bennett *et al*., 2010).

Cellular proliferation and differentiation in the root

Root cells develop along the root's longitudinal axis (Fig. 3A). Cell proliferation takes place in the root meristem, a zone that is situated in the root tip directly shootward of the root cap. The columella is the only tissue in which cells differentiate only rootwards (in the direction of root growth). All other tissues are formed by cells that differentiate shootwards (in the direction opposite to root growth). In the course of their differentiation, root cells rapidly expand longitudinally, thereby pushing the root tip along the growth direction of the root. Clonal analyses traced the origin of all root cells back to root initials (Dolan *et al*., 1994; Scheres *et al*., 1994) that are organized around the QC (Fig. 3A). Laser ablation studies showed that in order to maintain stem cell-like status, the root initials need to be in contact with the QC cells (van den Berg *et al*., 1997). QC cell identity is linked to the expression of the *WUSCHEL-RELATED HOMEOBOX 5* (*WOX5*) transcription factor gene (Sarkar *et al*., 2007). The expression of *WOX5* is restricted to the QC cells by the presence of the *WOX5* repressor, ACR4, in the neighbouring cells. *ACR4* is a receptor-like kinase gene that is expressed in the neighbouring columella initials and columella cells (De Smet *et al*., 2008). *WOX5* repression by ACR4 is dependent on the signal peptide CLE40 (Fiers *et al*., 2005) that is expressed in differentiated columella cells and represents the signal perceived by ACR4 leading to a repression of *WOX5* and stem cell fate (Stahl and Simon, 2009). Therefore, QC identity and homeostasis are governed by the *WOX5–ACR4–CLE40* pathway. Several additional gene modules have also been shown to be involved in QC maintenance. *SCR* (Di Laurenzio *et al*., 1996), originally described as a regulator of radial patterning of the ground tissue (see above), is also necessary to maintain QC cells (Sabatini *et al*., 2003), and the cell cycle regulator *RETINOBLASTOMA-RELATED PROTEIN 1* (*RBR1*) acts downstream of *SCR* in this process (Wildwater *et al*., 2005). Besides these, PLETHORAs (PLTs), AP-2 type transcription factors, are also crucial for QC maintenance (Aida *et al*., 2004). Expression of *PLT* genes is regulated by the auxin maxima in the root meristem (Aida *et al*., 2004; Petersson *et al*., 2009). In turn, *PLT* genes regulate the expression of *PINFORMED* (*PIN*) auxin efflux genes (Blilou *et al*., 2005), thereby contributing to the maintenance of the auxin maxima. Apart from the *WOX5–ACR4–CLE40*, the *SHR/SCR/RBR* (Cruz-Ramírez *et al*., 2012) and the auxin–*PLT* pathways, other less well-characterized pathways including *TYROSYLPROTEIN SULFOTRANSFERASE* and *ROOT MERISTEM GROWTH FACTORS* are also required to maintain a functional QC (Matsuzaki *et al*., 2010; Zhou *et al*., 2010).

Balancing the proliferation and differentiation rate in the root is key in determining root growth as root elongation is largely determined by the number of cell divisions of stem cell progenitors and their subsequent cellular expansion (Beemster and Baskin, 1998). Key for regulating the proliferation of cells in the RAM are general cell cycle genes including members of *CYCLIN-DEPENDENT KINASES* and *CYCLINS* (Inagaki and Umeda, 2011). Multiple pathways impact cell cycle regulation including reactive oxygen species (ROS) (Tsukagoshi, 2012), DNA damage (Culligan *et al*., 2004; Cools *et al*., 2011; Spadafora *et al*., 2011) and plant hormones (Takatsuka and Umeda, 2014). Not only cell proliferation, but also cell elongation can be regulated by cell cycle regulators through regulation of polyploidy via endoreduplication, a DNA replication process which repeats G_1 and S phases without G_2 and M phases and is not accompanied by cell division (Kondorosi *et al*., 2000). Cells with a higher level of ploidy grow to a larger size, thereby leading to increased root elongation (Hayashi *et al*., 2013). The transition to the endoreduplication cycle is suppressed by auxin (Ishida *et al*., 2010). Various other pathways impact cell elongation, such as cell wall formation by cellulose synthase activity (Chen *et al*., 2010) and re-orientation of cellulose fibres (Anderson *et al*., 2010).

The most prominent role in setting the rate of proliferation and differentiation in the root is that of the cross-talk between two major plant hormones, auxin and cytokinin. Examination of mutants of auxin transporters showed that the correct localization and intensity of the auxin response maximum is necessary for regulating proliferation and differentiation (Sabatini *et al*., 1999). The localization of this auxin response maximum is, to a large part, determined by the auxin reflux loop in the root apex (Fig. 3B). This reflux loop is maintained through membrane-localized auxin transport proteins: AUXIN RESISTANT1 (AUX1), LIKE-AUX2 (LAX2), LIKE-AUX3 (LAX3) (Bennett *et al*., 1996; Swarup *et al*., 2001; Péret *et al*., 2012*b*; Band *et al*., 2014), P-GLYCOPROTEIN ABC transporter family members (Geisler and Murphy, 2006) and the PIN proteins (Blilou *et al*., 2005). In particular, PIN proteins are critical for polar auxin transport. While single *pin* mutants exhibit subtle phenotypes in the primary root (Gälweiler *et al*., 1998; Müller *et al*., 1998; Friml *et al*., 2002*a*, *b*, 2003; Blilou *et al*., 2005), most double mutants generated for *PIN1*, *PIN2*, *PIN3*, *PIN4* and *PIN7* show additive effects on the orientation of cell division, root meristem size and root length (Blilou *et al*., 2005). However, *pin1pin2* double mutants, and all triple and quadruple mutants containing *pin2*, display epistatic behaviour (Blilou *et al*., 2005). Overall, these data underscore the importance of PINs and polar auxin transport in patterning and setting the rate of proliferation and differentiation in the root. Consequently, the exogenous application of auxin leads to promotion of cell division and an increase in meristem size (Blilou *et al*., 2005; Dello Ioio *et al*., 2007); however, cell elongation is reduced, which altogether results in shorter roots (Rahman *et al*., 2007). In contrast to auxin, the exogenous application of

cytokinin causes a decrease in the root meristem size, while the cell division rate remains unchanged (Dello Ioio *et al*., 2007). Accordingly, cytokinin biosynthesis mutants have longer meristems (i.e. an increased number of cells from the QC to the first elongated cell), which is phenocopied by the constitutive expression of the cytokinin-inactivating enzyme AtCKX1 in the vasculature tissue at the transition zone. This indicates that cytokinins promote differentiation in the root meristem (Dello Ioio *et al*., 2007) while auxin promotes cell division. These opposing functions are crucial to determine the balance of proliferation and differentiation and therefore the location of the transition zone. At the molecular level, auxin and cytokinin interact by regulating in opposing ways the abundance of SHY2/IAA3 protein, a member of the auxin-induced Aux/IAA family (Dello Ioio *et al*., 2008). Cytokinin signalling mediates transcription of *SHY2* through the AHK3/ARR1 signalling pathway. In turn, SHY2 downregulates expression of the auxin transport genes *PIN1*, *PIN3* and *PIN7*. On the other hand, auxin promotes degradation of SHY2 via the SCF^{TIR1} pathway, which derepresses transcription of the cytokinin biosynthesis gene *IPT5* (Dello Ioio *et al*., 2008). Overall, this balance between auxin and cytokinin signalling sets the balance between cell division and cell differentiation at the root transition zone, thus regulating meristem size and consequently root growth rate. Besides these very well characterized interactions, many regulatory steps exist for cytokinin as well as auxin pathways. These are present at almost every level, including their biosynthesis, transport, signalling and metabolism, each of which has a major impact on root development. Therefore, to generate accurate models of hormonal action and interaction at the molecular level, such as for auxin and cytokinin in the root tip, knowledge of the cellular and sub-cellular distribution of signalling components and hormones is needed. Initial steps in these directions have been made. For instance, high-resolution maps of the intracellular distribution of auxin (Petersson *et al*., 2009) and cytokinin (Antoniadi *et al*., 2015) in the root apex have been generated. In the case of auxin, these data supported the relatively recent concept that auxin is not exclusively shoot derived but that local auxin biosynthesis substantially contributes to auxin homeostasis in the root tip (Ljung *et al*., 2001; Bhalerao *et al*., 2002; Petersson *et al*., 2009).

While much has been learned about the pathways governing stem cell homeostasis, and the balance of proliferation and differentiation in the past years (see also later), many questions remain, such as at which growth stage, in which cellular environment and in which environmental context different pathways actually contribute, which stimuli impinge on which pathways, and how specifically these pathways interact with each other.

Lateral root formation

In the seed, only the PR is present as the radicle in the plant embryo (Grunewald *et al*., 2007). Higher order roots are the result of post-embryonic LR formation events that represent *de novo* organogenesis (Dubrovsky *et al*., 2006). LR formation is a key process that significantly contributes to shape the RSA (Lynch, 1995*b*). In arabidopsis and most dicots, LRs are formed from pericycle cells, which are adjacent to the xylem pole (Casimiro *et al*., 2003; Péret *et al*., 2009). LRs are usually spaced along the PR in a regular left–right alternating pattern (De Smet *et al*., 2007). LR formation can be partitioned into several stages (Malamy and Benfey, 1997) and involves four key events: priming, initiation, primordium formation and emergence. Priming and specification of future LR primordium sites are correlated with increased auxin signalling in the basal meristem (De Smet *et al*., 2007; Moreno-Risueno *et al*., 2010). These primed LR sites are xylem pole pericycle cells. These are different from other pericycle cells and show downregulation of *Kip-Related Protein* genes (*KRP1* and *KRP2*), which are inhibitors of the G_1 to S transition (Himanen *et al*., 2002). Auxin accumulation in these 'founder cells' precedes LR initiation. Subsequent maintenance of these auxin maxima is necessary for proper LR organogenesis (Dubrovsky *et al*., 2008). The auxin maximum is regulated by auxin reflux between the endodermis and the pericycle, mediated by the auxin efflux carrier PIN3 (Marhavy *et al*., 2013). The resulting auxin gradient is necessary for the activation of a key module in LR priming, the *SOLITARY ROOT*/*ARF7*/*ARF19* genes (Fukaki *et al*., 2002; Vanneste *et. al*., 2005). This module activates transcription of *LBD16*/*ASL18* and *LBD29*/*ASL16*, whose activities are all required for LR formation (Okushima *et al*., 2007). After priming, an asymmetric cell division takes place, marking LR initiation. Here, the receptor-like kinase ACR4 was identified as a critical factor for ensuring the correct specification of the LR primordium (De Smet *et al*., 2008). A further auxin module, *BODENLOS*/*IAA12-MONOPTEROS*/*ARF5* is another key module during this stage of LR formation (De Smet *et al*., 2010). All these events occur in the pericycle, an internal layer of the root. Consequently, the newly formed LR primordium has to break through the ground tissue (endodermis, cortex and epidermis layers). This emergence process is highly regulated and, in particular, LR primordium-derived auxin is crucial for reprogramming adjacent cells. The co-ordinated action of the auxin influx carriers AUX1 and LAX3 plays a key role in this (De Smet *et al*., 2007; Swarup *et al*., 2008). Specifically, the auxin-induced expression of *LAX3* in the cortical and epidermal cells that directly border the new primordium leads to the induction of cell-wall-remodelling enzymes that facilitate proper loosening and separation of the overlaying layers during the emergence of the newly formed LR (Swarup *et al*., 2008). During this process, direct communication of the pericycle and the endodermis, involving SHY2-mediated auxin signalling, is crucial for LR initiation (Vermeer *et al*., 2014).

While much of the LR formation is driven by auxin, other hormones impact LR formation, mainly by modulating auxin-dependent processes. Similar to the RAM, cytokinins act antagonistically to auxin during LR initiation by modulating auxin transport and preventing the formation of auxin maxima (Laplaze *et al*., 2007). Consequently, repressed cytokinin responses were observed in the founder cells, while enhanced cytokinin responses took place in the pericycle cells between two existing primordia (Bielach *et al*., 2012). Additionally, younger LR primordia are more sensitive to perturbations in the cytokinin pathway compared with those in later developmental stages (Bielach *et al*., 2012). Cytokinin biosynthetic genes play an important role in suppressing the initiation of new LRs in the neighbouring cells. Mutations of these genes caused a reduction of cytokinin in the cells adjacent to the LR primordium, which

led to an abnormal positioning of LRs in an *ACR4*-independent fashion (Chang *et al*., 2015).

Abscisic acid (ABA) is another hormone that modulates LR formation through auxin signalling. ABA inhibits LR formation immediately after LR emergence in a reversible manner (De Smet *et al*., 2003). The *ABI3* (*ABA INSENSITIVE3*) member of the ABA signalling pathway is induced by auxin in the LR primordium, and loss-of-function *abi3* plants show reduced response to auxin and auxin transport inhibitors (Brady *et al*., 2003). Another key transcription factor linking auxin and ABA is MYB77. This transcription factor interacts with ARF7 (Shin *et al*., 2007) and PYL8, an ABA receptor (Zhao *et al*., 2014).

Lateral root formation, and therefore root architecture, is strongly impacted by nutrient availability (Lopez-Bucio *et al*., 2003). For example, LR emergence of arabidopsis grown on high nitrate concentrations is systematically inhibited (Walch-Liu *et al*., 2006). In contrast, a local increase of nitrate concentration has a stimulatory effect on LR growth, which is abolished in the auxin-resistant mutant *axr4* (Zhang *et al*., 1999). Several other molecular links between nitrate and auxin pathways in regulating LR development were identified (Guo *et al*., 2002; Gifford *et al*., 2008; Vidal *et al*., 2010; Rosas *et al*., 2013). Strikingly, the nitrate transporter NRT1.1 also facilitates auxin transport in the LR primordium, thus controlling root branching (Krouk *et al*., 2010). When nitrate is absent, NRT1.1 blocks auxin accumulation and growth of the LR by supporting basipetal auxin transport. At higher nitrate concentrations (e.g. 1 mM), the action of NRT1.1 is blocked, leading to accumulation of auxin in the LR primordium and permitting its growth (Krouk *et al*., 2010). Nitrogen interferes with other hormonal pathways as well. For instance, nitrate application induces the expression of genes involved in cytokinin biosynthesis (Sakakibara *et al*., 2006), which are important for proper LR patterning (Chang *et al*., 2015). Nitrate and ABA pathways are connected by *ABI4* and *ABI5*, as the two ABA-insensitive mutants lines *abi4* and *abi5* display a lesser degree of reduction of LR formation by high nitrate concentration (Signora *et al*., 2001). Phosphorus is another example of how nutrients affect hormonal signalling in order to modulate LR formation, and thereby RSA. In phosphate-deficient conditions, expression of the auxin receptor gene *TRANSPORT INHIBITOR RESPONSE1* (*TIR1*) is stimulated, which in turn promotes the activation of downstream ARF transcription factors that are involved in the control of LR formation (Pérez-Torres *et al*., 2008). Finally, iron-dependent LR growth promotion is dependent on rootward auxin transport. In particular, the auxin influx carrier AUX1 modulates this response, since iron-stimulated LR elongation is lost in *aux1* mutants (Giehl *et al*., 2012)

Control of root growth direction

The direction in which a root grows is a key parameter for soil exploration and the response to environmental cues. The directional characteristics of growth are termed tropisms and, depending on whether plants grow towards a signal or away from it, the tropism is defined as positive or negative, respectively. There are multiple tropisms including gravitropism (gravity), phototropism (light), hydrotropism (water) and thigmotropism (touch) (Esmon *et al*., 2005).

Gravitropism is highly regulated by auxin transport mechanisms (Baldwin *et al*., 2013). Polar localization of PIN proteins creates an asymmetrical auxin distribution and allows gravitropic bending by elongation of only the side of the root that is furthest from the direction of the vector of gravity (Wisniewska *et al*., 2006). Other genes that have been shown to be important regulators for the gravity response include the DnaJ-like protein gene *ALTERED RESPONSE TO GRAVITY* (Sedbrook *et al*., 1999), an E3 ligase family gene *WAVY GROWTH 3* (Sakai *et al*., 2012) and *PHOSPHOLIPASE C* (Andreeva *et al*., 2010). In comparison with gravitropism, molecular mechanisms of other root tropisms are not well understood. Root phototropism has been known for a long time. While usually not directly exposed to light, roots are frequently exposed to light through ambient diffusion or soil upheaval (Galen *et al*., 2007). Moreover, photons can be efficiently conducted through the vasculature (Sun *et al*., 2003). The occurrence of phototropism is species dependent, and it has been reported that almost 43 % of examined plant species showed a negative root phototropism (Kutschera and Briggs, 2012). A blue light receptor gene *NONPHOTOTROPIC HYPOCOTYL 1*/*PHOTOTROPIN 1* (Liscum and Briggs, 1995) and red light receptor genes *PHYTOCHROME A* and *B* (Kiss *et al*., 2003) are involved in negative and positive root phototropism, respectively. Examples of molecular mechanisms and genes involved in hydrotropism are, for instance, the cytokinin-dependent involvement of *ALTERED HYDROTROPIC RESPONSE 1* (Saucedo *et al*., 2012), the ABA-dependent involvement of *NO HYDROTROPIC RESPONSE 1* (Ponce *et al*., 2008) and the land-plant-specific gene *MIZU-KUSSEI 1* (*MIZ1*) (Kobayashi *et al*., 2007). Overall, it is thought that these components modulate how the root cap senses and responds to water availability (Cassab *et al*., 2013). *MILDEW RESISTANCE LOCUS O4* (Chen *et al*., 2009) and *ENDOBINDING 1* (Gleeson *et al*., 2012) have been identified as thigmotropism regulators that when mutated, displayed intense responses to touching surfaces.

Since multiple stimuli occur simultaneously in nature, it is expected that multiple tropism mechanisms impact each other. While the signalling mechanisms of different tropisms are independent [for instance the absence of hydrotropism in *miz-1* mutants did not have any effect on gravitropism (Kobayashi *et al*., 2007)], there is significant cross-talk. For instance, gravitropism and hydrotropism affect each other (Takahashi *et al*., 2009), and, while hydrotropism of arabidopsis is dominant to gravitropism (Takahashi *et al*., 2002), that of pea roots is overcome by gravitropism (Takahashi *et al*., 1992). Overall, this shows that multiple signals coming from largely independent signalling pathways are integrated, but which tropism will dominate depends on the genetic constitution of the plant.

APPROACHING COMPLEXITY IN ROOT GROWTH CONTROL USING SYSTEMS BIOLOGY

With the discovery of an ever-increasing number of genes that are involved in regulating root growth and their complex interactions, it became clear that more holistic approaches were needed to comprehend the regulation of root growth. An area of biology that has emerged to approach such challenges is

systems biology. Here, neither single genes nor proteins are at the focus of the research; instead, the focus is on the systems that are defined by interactions of biologically relevant entities such as genes, proteins, cells, tissues, organs or organisms. The study of root growth in arabidopsis has long been at the forefront of plant systems biology (Hill *et al*., 2013). We will highlight multiple areas in which it has significantly expanded our comprehension of complex biological processes that are important in the context of root growth and development.

Understanding networks at the level of cell types

One of the first milestones in the plant systems-biology field was the generation of a cellular atlas of gene expression by combining fluorescence-activated cell sorting (FACS) of green fluorescent protein (GFP)-labelled cell populations and microarray analysis (Birnbaum *et al*., 2003). This allowed, for the first time in plants, an insight into cell type expression patterns of the >20 000 genes that were present on the ATH1 microarray platform. In the subsequent years, this expression atlas was refined by including more cell types and distinct developmental zones of the root (Brady *et al*., 2007), as well as by applying stress to the roots before FACS (Dinneny *et al*., 2008; Long *et al*., 2010; Iyer-Pascuzzi *et al*., 2011). Together, these large-scale approaches enabled the discovery of cell-type-specific dominant expression patterns of large numbers of genes, as well as the discovery of stress response centres and the impact of stress on cell fate decisions (Dinneny *et al*., 2008; Long *et al*., 2010; Iyer-Pascuzzi *et al*., 2011). Importantly, these approaches and tools, due to their easy accessibility (Brady *et al*., 2007; Winter *et al*., 2007), constituted highly useful resources for a whole field, enabling rapid insights into gene expression patterns. Overall, they have paved the way for popularizing *in silico* gene expression analysis and have also provided a valuable resource for identifying novel molecular mechanisms and regulatory models. For instance, screening for transcription factors that were specifically expressed in the transition zone, where cell proliferation ceases and cell differentiation starts, resulted in the discovery of *UPB1* and a novel mechanism regulating the balance of ROS between the zones of cell proliferation and the zone of cell elongation where differentiation begins (Tsukagoshi *et al*., 2010). Indeed, the transcriptional regulation of ROS by *UPB1* controls the transition from proliferation to differentiation in the root (Tsukagoshi *et al*., 2010). A novel mechanism for the periodic patterning of the root was also discovered based on the Brady transcriptome data. In particular, transcriptome data from two individual roots were subjected to methods capable of identifying periodic responses. The results of this analysis, together with long-term imaging, revealed that the position of LRs and root bending are periodic responses, which appear to be regulated by a mechanism resembling an endogenous clock (Moreno-Risueno *et al*., 2010).

Motivated by such successes, cell-type-specific atlases have been created for other molecules such as small RNAs (Breakfield *et al*., 2012), proteins (Petricka *et al*., 2012) and metabolites (Moussaieff *et al*., 2013). However, while profiling of mRNA is relatively straightforward, the other atlases faced technical difficulties, such as the large number of cells required for metabolite measurements or the incomplete sampling of the proteome by GeLC-MS/MS (in-gel tryptic digestion followed by liquid chromatography-tandem mass spectrometry).

The root gene expression atlas has also been the starting point for generating gene network models that try to infer relationships of gene regulation. For instance, transcription factors and miRNAs that were preferentially expressed in the stele were subjected to systematic yeast one-hybrid (Y1H) and Y2H assays to assess the binding of transcription factors to transcription factor and miRNA-promoters and the interaction between transcription factors at the protein level (Brady *et al*., 2011). These interaction data, in conjunction with expression data, were then used to generate a directed network model. The accuracy of this model was verified by testing predicted interactions using chromatin immunoprecipitation (ChIP) for transcription factors and predicted direct targets, as well as expression and phenotypic analysis of mutant lines of targets. Interestingly, 65 % of the transcription factor knockout lines tested showed molecular phenotypes, but only 16 % showed morphological phenotypes (Brady *et al*., 2011). Overall, these results highlighted the robustness of biological regulation and the difficulty in studying complex gene regulation at the systems level using only single-gene knockout lines. Along similar lines, a network model of secondary cell wall synthesis was recently generated (Taylor-Teeples *et al*., 2014). For this, genes were selected using the root expression atlas as well as annotations; molecular interactions of these genes were then mapped by Y1H, and a network constructed from all of the data. This network model of secondary cell wall synthesis provided specific insights into the gene regulation underlying cell wall synthesis, as well as its modulatory capacity under stress conditions (Taylor-Teeples *et al*., 2014).

Overall, these recent studies show the power of cell-type-resolved experimental data sets to uncover complex regulatory relationships between large sets of genes, yielding models with predictive power.

Understanding specific regulatory networks

While cell-type-specific gene expression maps allow for the identification of genes and the networks and pathways that they act in, similar inferences can be made from transcriptome data sets that are centred around specific genes or regulatory processes. For instance, a cell sorting approach combined with a transcriptome analysis of the LR regulator S-phase kinase-associated protein 2 (SKP2B), followed by the identification and analysis of genes that were enriched in SKP2B-expressing cells, revealed an important role for redox-related genes and ROS-dependent mechanisms in LR development (Manzano *et al*., 2014). Transcriptome analyses identified GATA23 as a key factor defining LR founder cell identity (De Rybel *et al*., 2010), and the receptor-like kinase ACR4 was identified as a key regulator for the formative cell divisions of LR organogenesis (De Smet *et al*., 2008). A transcriptome approach analysing FACS-sorted root hair epidermal and non-root hair epidermal cells from multiple mutant lines displaying either more or fewer root hairs than the wild type, in conjunction with a modelling approach, generated a comprehensive network model of 208 core epidermal genes (Bruex *et al*., 2012).

Studies analysing time courses of transcriptome data acquired after induction of biological or developmental processes have great power to resolve complex biological processes at the molecular level. For instance, chemical induction of LR formation revealed specific transcriptional stages that precede cell divisions in the process of LR formation (Himanen *et al*., 2004). A similar study identified 60 distinctly responding genes clusters, and underscored cell wall remodelling as an essential feature of LR development (Lewis *et al*., 2013). A high-resolution time course experiment after root bending revealed that a large number of genes showed an oscillatory pattern during LR formation. Further analysis of these data suggested that the circadian clock was rephased during LR formation and that this process is necessary for gating auxin signalling during LR development to facilitate organ emergence (Voß *et al*., 2015). The same time course data were reanalysed with a custom time-delay correlation algorithm (TDCor) to generate a comprehensive gene regulatory network (GRN) in order to identify genes and their interactions controlling LR primordium initiation and patterning. Notably, this GRN model revealed that cell fate decisions during early LR patterning are dependent on the mutual inhibition between the *ARF7* and *ARF5* regulator modules (Lavenus *et al*., 2015). Another study in the context of cell fate decisions in the RAM used dense time course transcriptome data coupled with genome-wide detection of direct targets for *SHR* to uncover the temporal dynamics of regulation of the formative cell division of the CEI, including the discovery of a specific cell cycle gene (*CYCD6;1*) of crucial importance for this particular cell division (Sozzani *et al*., 2010).

Such data can be used as an excellent starting point for capturing complex regulatory processes in space and time via mathematical modelling approaches. For instance, based on the regulation of *CYCD6;1* by SCR/SHR and a potential link to the RBR protein, an iterative approach combining genetics, cell biology and mathematical modelling was able to identify the specific molecular interactions that determine the precise spatio-temporal pattern of the CEI formative cell division (Cruz-Ramirez *et al*., 2012). Key to this success was a procedure in which predictions of alternative mathematical models of interactions of multiple key components were compared with experimental results (Cruz-Ramirez *et al*., 2012). Another highly successful approach that combined genetic analysis and mathematical modelling was the identification of the genetic network and molecular mechanism that generates distinct hormonal response zones in the vasculature; the results sufficiently explained vascular tissue growth and patterning (De Rybel *et al*., 2014).

Dissecting the complex networks of plant hormonal signalling

Plant growth and development are co-ordinated and controlled by highly complex hormonal signalling pathways that act locally as well as systemically. The intrinsic complexities of these pathways are difficult to comprehend by reductionist approaches. Modelling approaches have turned out to be particularly useful in approaching this complexity. A landmark study in modelling hormonal signalling in the root was a model of auxin distribution that incorporated diffusion and PIN-facilitated auxin transport across cells using a simplified root layout. It was able to recreate experimentally observed auxin distribution patterns and morphogenesis over a broad range of time scales (Grieneisen *et al*., 2007). Further refinement was achieved by incorporating real-world root cell geometries and experimentally determined localization of auxin transporters into the model. Experimentally tested using the DII-VENUS auxin sensor and computational image analysis, this model led to the insight that auxin efflux carriers alone cannot generate the experimentally observed auxin distribution at the root tip. Rather, non-polar auxin influx transporters control auxin abundance, and the polar PIN efflux transporters control the direction of auxin transport in these tissues (Band *et al*., 2014). An auxin signalling reporter and mathematical modelling were used to quantify auxin redistribution after a gravitropic stimulus. Interestingly, auxin was redistributed very rapidly and returned to the normal distribution after the root tip had changed its angle to 40 ° to the horizontal. This led to the postulation of a tipping point mechanism that reverses the auxin flow after bending of the root has reached an angle threshold (Band *et al*., 2012). Most recently, an iterative mathematical modelling approach that incorporated diverse parameters such as growth, gene expression, protein turnover and movement, auxin levels and response, and experimental testing of the model's predictions, led to insights into the question of how auxin can concurrently mediate rapid responses such as gravitropism, and yet regulate stable developmental zonation (Mähönen *et al*., 2014).

The complex interplay of developmental regulation by auxin and RSA was illustrated by a model which combined cell shape and auxin transport, revealing that the local curvature of the root has an intricate impact in mediating changes in auxin transport and therefore local concentration (Laskowski *et al*., 2008). A mathematical modelling approach that took into account three-dimensional cell and tissue geometries, as well as expression of auxin transporters and their transport activity, revealed the necessity for a particular activation sequence of the LAX3 auxin influx carrier and PIN3 auxin efflux carrier for LR emergence (Péret *et al*., 2013). Using a mathematical model describing the biophysical properties of the root, in particular the LR primordium, as well as the effects of water flows mediated by the PIP2;1 aquaporin, important predictions of LR emergence in *pip2;1* mutants and overexpressors were confirmed, showing the importance of the spatial and temporal aquaporin-dependent transport of water through root tissue (Péret *et al*., 2012*a*).

While these studies had been focused largely on auxin distribution in the root, another study focused on the impact of cell identity on auxin-dependent gene expression responses. Here, transcriptome analysis in four distinct tissues of the arabidopsis root demonstrated that tissue identity and developmental stage made important contributions to auxin responses, with auxin-dependent genes showing down- or upregulation depending on the tissue context (Bargmann *et al*., 2013). Modelling even more specific aspects of molecular auxin signalling components, such as the Aux/IAA system, predicted that the ratios between protein and mRNA turnover rates are key determinants of the systems properties (Middleton *et al*., 2010).

While auxin signalling has received the most attention, other hormonal signalling pathways have also been investigated. Two examples include insights into the distinct time scales and feedback loops of different components involved in gibberellin signalling (Middleton *et al*., 2012), and the importance of

receptor complex oligomerization states of the ABA receptor for modulating hormonal responses (Dupeux *et al*., 2011). Moreover, the interplay between different hormone pathways was also studied. Modelling of a regulatory circuit centred around *POLARIS* (*PLS*), which mediates cross-talk between auxin, ethylene and cytokinin in arabidopsis, revealed that this cross-talk is mainly shaped by PLS controlling the relative contribution of auxin transport and biosynthesis (Liu *et al*., 2010). Overall, systems-biology approaches have increased our comprehension of complex regulatory systems far beyond the level of what could have been achieved using traditional forward genetic approaches.

OUTLOOK: MOVING TOWARDS ROOT SYSTEMS GENETICS

Systems-biology approaches are highly successful in shedding light on complex biological processes. However, there are significant limitations for many of these methods for capturing dynamic processes. For instance, while time courses of transcriptome data are becoming more common, there are still limits with regard to how many time points can be acquired, especially with cell type resolution (partly due the cost of these approaches and partly due to technical reasons). Moreover, transcriptome approaches measure only transcriptional responses, and important regulatory levels such as protein abundance or protein localization changes cannot be captured using these approaches. With many other data types with less spatial resolution, such as most protein–DNA binding profiles (ChiP-chip/ChIPseq), or data acquired in yeast such as protein–protein (Y2H), and promoter–transcription factor interactions (Y1H), it is often not clear how to interpret these data in the context of a multicellular organism with processes occurring over time in different cell and tissue contexts.

In contrast, phenotypic analysis of mutant lines or natural accessions can provide very high spatial and temporal resolution that can be acquired in various conditions, but it cannot be immediately related to complex molecular processes. However, recent advances have made it possible to link complex molecular data with complex phenotypic data. In particular, large-scale phenotyping approaches in combination with genome-wide association mapping [genome-wide association studies (GWAS)] allow for the mapping of complex and highly resolved phenotypes to the whole genome, and to identify the loci that determine these phenotypes (Fig. 4). If combined with systems-biology-type data, GWAS provide the possibility to study how genetic information is translated by molecular, cellular and physiological networks to shape complex phenotypes (Nadeau and Dudley, 2011). Such efforts have been defined as the subject of the emerging field of systems genetics (Ayroles *et al*., 2009; Mackay *et al*., 2009; Nadeau and Dudley, 2011; Civelek and Lusis, 2014). Systems genetics is a synthesis of multiple fields, including genetics, genomics, systems biology and phenomics (Markowetz *et al*., 2015). Much like systems biology, it aims to study the relationships of multiple components and how interactions of these components give rise to biological function. However, systems genetics specifically aims to address the question of how genetic variation leads to phenotypic (trait) variation. This promises to approach similarly complex questions to those approached using systems biology, but firmly anchors these approaches in the genotype to phenotype problem, thus adding a genetic and phenotypic/physiological axis. Likewise, it extends traditional genetics since it adds functional insights into intermediate layers between genotype and phenotype. Moreover, due to the ability to measure many phenotypes of the same genotypes in many conditions, such approaches are not restricted to one trait but are ideally extended to multiple traits, their dependence on each other, and their interaction with

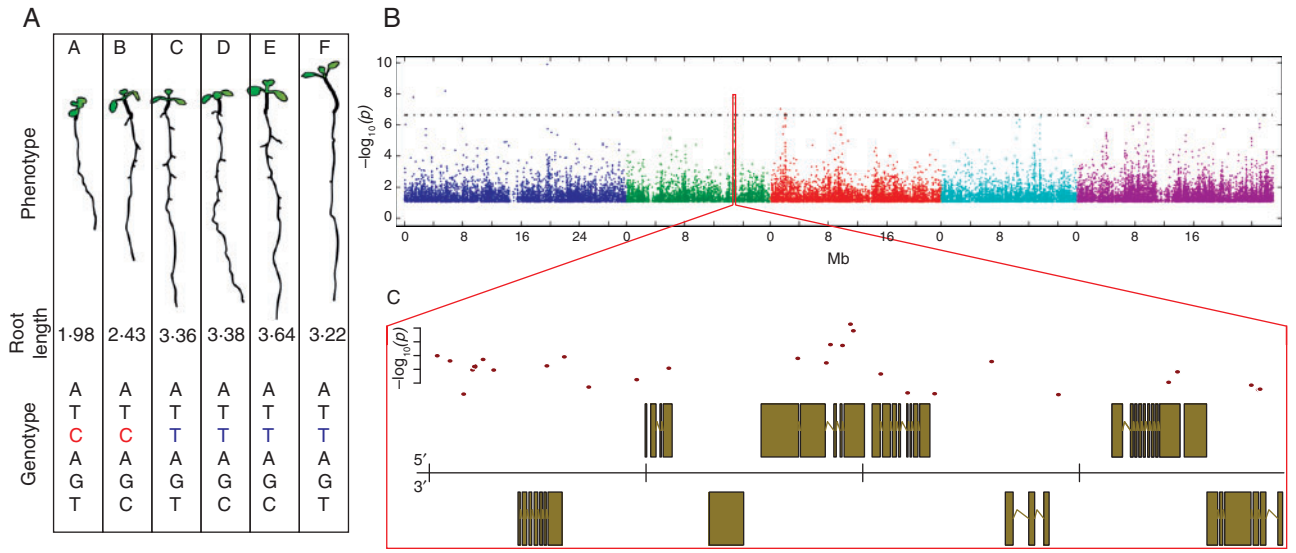

FIG. 4. Schematic of an exemplary GWAS. (A) Phenotypes of various genotyped strains (upper part) are quantified for a trait (in this example root length; mid-part). GWAS is conducted by using the quantified phenotypes and their genotypes to find significant associations of single nucleotide polymorphisms (SNPs) and the phenotype. Exemplary sequences that highly associate with the example root length values are shown in the lower part. (B) Example of a Manhattan plot that depicts the genome-wide associations {*x*-axis, SNP position along the genome; *y*-axis, significance of association [$-\log_{10}$(*P*-value)]; associations coloured by chromosome}. (C) Genomic region surrounding a significant GWA peak. Top: $-\log_{10}$(*P*-values) of associations of the SNPs. Bottom: gene models in genomic regions. The *x*-axis represents the position on the chromosome.

the environment. For instance, a systems-genetics approach can unravel which molecular consequences of a particular genotype, under certain environmental conditions, lead to changes at the cellular level and how these cellular changes relate to specific organismal phenotypes. This would lead to not only the prediction of molecular components that would alter these phenotypes when targeted by breeding, specific drugs or treatments, but also to mathematical models predicting phenotypes from genotypes. While systems genetics is still in its infancy, there has been recent progress in this direction in the root. For instance, a recent study systematically assessed cellular root traits in 201 arabidopsis accessions. This led to the identification of strong correlations between different cellular traits such as meristem size and mature cell size, indicating a tight genetic control of proliferation and differentiation in the root. Using expression data for the genes in the associated genomic region, it was possible to identify the causal gene and its alleles for the two highly correlated root cellular traits (Meijón *et al*., 2013). Importantly, these cellular changes translated into changes in root length. Overall, this demonstrated that by combining GWAS and expression information, it was possible to identify an unknown regulator and its alleles that regulate two highly correlated cellular traits, thereby regulating root growth, an organ-level phenotype. Another study approached the relationship between traits, growth conditions and genotypes using GWAS, genome-wide expression analysis and phenomics. This study not only uncovered the fact that most root traits, such as the length of primary and LRs or LR density, are independently controlled in a genotype- and environment-dependent manner, but also mapped a large number of potentially causal genes underlying this remarkable plasticity (Gifford *et al*., 2013). Due to the increasing number of highly efficient phenotyping pipelines for roots, the growing popularity of GWAS for root traits and the excellent functional genomics resources for roots, much more progress in this area can be expected.

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