

# Rooting plant development

Ben Scheres\*

## Summary

In 1993, we published a paper in *Development* detailing the anatomical structure of the *Arabidopsis* root. The paper described how root growth was maintained by the precisely tuned activity of a small set of ‘initials’, which acted as the source of dividing and differentiating cells, and how these stem cell-like cells surrounded a few infrequently dividing cells. This work underpinned subsequent research on root developmental biology and sparked a detailed molecular analysis of how stem cell groups are positioned and maintained in plants.

## Before we knew about *Arabidopsis* roots

At the time when the molecular genetic analysis of animal development was already at its peak, plant developmental biology was mostly a descriptive science that merged anatomical studies with elegant physiology. I remember being awestruck by the precision with which developmental processes could be dissected in organisms such as *Caenorhabditis elegans*, and wanted to find a ‘plant worm’. Around 1990, it was becoming clear from beautiful work on flower development and embryogenesis, initiated by former *Drosophila* geneticists, that the genetically tractable model *Arabidopsis thaliana* was the plant of choice to study developmental biology. Key concepts that had just emerged from these studies were that combinatorial coding by homeotic genes served to specify organ identity, and that genetic screens could reveal mechanisms of embryonic development, both concepts being reminiscent of earlier findings in *Drosophila* development. These new studies challenged several traditional paradigms in plant biology. For example, clonal analyses suggested that positional cues are all-important in plant development, but the new genetic screens emphasized the importance of ‘genetic addresses’ in regions and, similar to *C. elegans*, the apparent constancy of lineage relationships. Seeing these advances, I focused on the root because textbook botany knowledge indicated that all plant roots are anatomically simple, which would hopefully allow us to investigate lineage and positional cues both by genetics and by experimental manipulation, as had already been done in the animal field.

At that time, I had an interesting encounter at the fourth international *Arabidopsis* conference in Vienna. Somebody told me that a laboratory with some interest in root development had looked into *Arabidopsis* root meristems – the growth tips of roots containing the dividing cells that build the organ. He recalled that these investigators did not find a well-organized root meristem at all, and that this might be due to the fact that the short-lived *Arabidopsis* plant, being able to produce seeds in 6 weeks, did not really need a long-term reservoir of cells to fuel root development. That was an unsettling message, as I had just seen cross-sections of the *Arabidopsis* root with beautifully constant symmetries that reminded me of the almost invariant division patterns in *C. elegans*. Fortunately, instead of wandering off in other directions, I decided to ignore these warning signs.

## The paper

Although our methods to describe *Arabidopsis* root development were extremely traditional – a combination of conventional microscopy techniques – they were not trivial because of the difficulty in straightening out the slender structure of the *Arabidopsis* root. It was easy to miss the elementary and simple organization at the tip of the root in a tilted focal plane, and we figured this was the reason that the structure had not been noted before. And how beautiful it was! Looking at micrographs it became clear that the root was built by intricately controlled divisions, yielding reproducible tissue arrangements. The organizational core could be described in a simple summary picture and had all the features of the structures later called stem cell niches (Fig. 1).

On a very pleasant visit to Norwich I discovered that we were not the only ones that noted the regularity of root development. Liam Dolan came from a history of leaf clonal analysis and wanted a system to look at ‘simple’ fate decisions, so he had carried out genetic screens to identify genes controlling cell fate in the root epidermis. Aided by the vast microscopy expertise in Keith Roberts’ group, he found that the extremely regular pattern of hair and non-hair cells in the epidermis could be traced back into the meristem. Their analyses of division patterns and cell morphologies were complementary to ours and we decided there and then to publish this work together. More work on electron microscopy descriptions of main and lateral root development from their side, and DNA labeling to pinpoint a small kernel of central cells that divided more rarely from ours, completed the story.

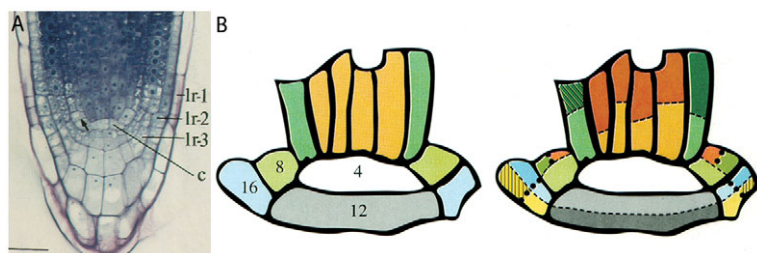
The paper that was sent to *Development* was entirely descriptive (Dolan et al., 1993), yet it was clear to the editor at the time, Chris Wylie, that it could serve as the foundation for a body of future work. He mostly asked us to reduce the specific botanical jargon to give readers outside the field a chance to digest the paper. Indeed, a wealth of questions immediately came from the description of the core set of cells that so precisely laid down a patterned organ, the root. Did lineage or position determine cell identity? Was it coincidental that the anatomy appeared to follow a blueprint of the tissue organization in the embryo? What was the nature of the ‘initials’, later equated to somatic stem cells, and what was the function of the quiescent center? What controlled the precisely

### A *Development* classic

The year 2012 marks 25 years since the journal *Development* was relaunched from its predecessor, the *Journal of Embryology and Experimental Morphology (JEEM)*. In 2008, we fully digitised our *Development* and *JEEM* archives, and made them freely available online. At the same time, we took the opportunity to revisit some of the classic papers published in *JEEM*, in a series of commentaries (see Alfred and Smith, 2008). Now, to mark a quarter century of *Development*, we have been looking through our archives at some of the most influential papers published in *Development's* pages. In this series of Spotlight articles, we have asked the authors of those articles to tell us the back-story behind their work and how the paper has influenced the development of their field. Look out for more of these Spotlight papers in the next few issues.

Plant Developmental Biology, Wageningen University Research, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

\*Author for correspondence (ben.scheres@wur.nl)



**Fig. 1. Stem cell organization of the *Arabidopsis* root as depicted in the original paper by Dolan et al. (A)** Transverse section with arrow indicating stem cell division and 'c' indicating one of the central cells later known as organizer cells; lrc-1, lrc-2, lrc-3 are lateral root cap layers. **(B)** Schematic of central cells and initials, later known as the stem cell niche, indicating cell numbers of each layer (left) and division planes (right).

oriented cell division planes? How was this structure built again during lateral root development?

At the time when this study was completed, Philip Benfey and John Schiefelbein, both in the USA, already appreciated the small number of cell and tissue types in *Arabidopsis* roots and the regular developmental transitions and had begun to exploit the recently established power of insertion genetics to describe the first mutants with root-specific defects, which now could be interpreted using the meristem and root structure as a guide (Schiefelbein and Benfey, 1991; Benfey et al., 1993). When these two scientists organized the first international *Arabidopsis* root meeting at New York University, there was great excitement about the potential of this system and long-lasting friendships were established among the early-day root aficionados.

## A wealth of questions immediately came from the description of the core set of cells that so precisely laid down a patterned organ, the root

### The impact of description

Several cornerstones of current-day plant developmental biology can be traced back to the description of the *Arabidopsis* root meristem.

First, this work gave rise to the notion of stem cells surrounding an organizing center of cells with low division activity. The role of these organizing cells could be probed experimentally using laser ablation due to the minimal size of the root (van den Berg et al., 1997). Building on this idea of specialized cells that maintain surrounding stem cells, a similar organizing center was described in the shoot apical meristem, when the expression domain of the WUSCHEL transcription factor was matched with its function in shoot meristem maintenance (Mayer et al., 1998). Gratifyingly, 10 years later this functional similarity was bolstered by the finding of a WUSCHEL homolog in the root organizing cells (Sarkar et al., 2007).

Second, the regular tissue layout described for the *Arabidopsis* root allowed the search for, and identification of, highly specific enhancer traps and specific promoters fused to GFP (Haseloff et al., 1997; Lee and Schiefelbein, 1999; Wysocka-Diller et al., 2000). These lines could be combined with the first sensor of the signaling activity of the plant hormone auxin to reveal the existence of a polar-auxin-dependent auxin maximum in the stem cell area of the *Arabidopsis* root (Sabatini et al., 1999). Auxin accumulation patterns have since been related to virtually every developmental process in plants that involves the definition of regions of growth. It has also become clear that these patterns are associated with the remarkable versatility of roots to regenerate tissue patterns after damage (Xu et al., 2006).

Third, a fine-grained molecular genetic description of root hair patterning and development was enabled by the known ontogeny

and spatial relationships between hair and non-hair cells (Masucci and Schiefelbein, 1996; Lee and Schiefelbein, 1999). Satisfyingly, similar but subtly different protein complexes turned out to be involved in trichome patterning (Kirik et al., 2004). These studies and those on factors involved in root hair differentiation (Menand et al., 2007) enabled new insights into evolutionary trajectories toward cell fate specification in entirely different organ contexts.

Fourth, the molecular analysis of endodermis and xylem patterning revealed remarkable roles for protein movement between plant cells in pattern formation. The best-studied example is the SHORTROOT pathway, in which a mobile transcription factor undergoes directional movement and subsequent nuclear localization to control an asymmetric cell division (Helariutta et al., 2000). In this case, as well as in the analysis of root hair patterning, cross-regulatory feedbacks have emerged that involve communication between cells through mobile proteins and miRNAs (Savage et al., 2008; Carlsbecker et al., 2010).

Together, these four research areas have bolstered the idea that, despite the very constant lineage relationships in the root, a continuous exchange of positional information underlies much of the regular architecture that was described some 20 years ago.

The simple organization of the root meristem facilitated several technical innovations that are widely exploited by the research community. It is worth mentioning one simple and one high-tech improvement. Tedious microscopy of small fixed root tips was replaced by direct imaging of the living meristem using the confocal laser-scanning microscope, allowing the visualization of live meristems at cellular resolution. This required vital root staining with propidium iodide, a method that resulted from a search for stains to monitor the effect of laser ablation in plants (van den Berg et al., 1995) and now used widely in the *Arabidopsis* field, allowing easy access to root structure for developmental and cellular analyses. A more recent revolutionary innovation combined protoplasting of roots, cell sorting and transcriptome analysis from isolated cells. This has allowed the creation of a digital *in situ* hybridization atlas of an organ, allowing the monitoring in unprecedented detail of gene expression changes over time or upon a range of stimuli (Birnbaum et al., 2005).

It seems to me that the combination of fundamental insights and technical advantages offered by the simple *Arabidopsis* root has made this an influential article and hence sparked the invitation to write this Spotlight. Although the basic description of the *Arabidopsis* root has found its way into many textbooks, the original Dolan et al. paper continues to be cited after almost 20 years – something that we secretly hoped back then, but certainly could not take for granted.

### Where are roots heading?

The recent literature shows that many of the initial questions that jumped out at us from the description of the *Arabidopsis* root are still under active investigation using this model system, albeit often framed in different language and loaded with new descriptors of

components and technologies unknown 20 years ago. To understand patterning and growth of the primary root, the initial observations of the auxin maximum have been connected to transcription factors operating in gradients (Galinha et al., 2007). Understanding their role in stem cell specification and root growth is likely to provide much more mechanistic models of organ growth. Another body of work describes roles for virtually all plant hormones and additional factors in root growth, which will allow us to understand hormonal cross-talk at the tissue and cellular levels (Dello Ioio et al., 2008; Matsuzaki et al., 2010; Tsukagoshi et al., 2010). After the initial description of *Arabidopsis* lateral root initiation, a series of papers have adopted the latest genetic, genomic and computational biology tools to investigate root branching, a pivotal topic bearing promise for agricultural innovation in a changing world. This research has led to several exciting non-exclusive models for lateral root patterning (De Smet et al., 2007; Laskowski et al., 2008; Moreno-Risueno et al., 2010).

## Perhaps the most exciting future direction resides in the broad evolutionary comparison of mechanisms discovered in roots to those operating in other contexts

Moreover, basic research on the mechanisms of cell polarization and control of division planes continues to exploit the experimental amenability of the *Arabidopsis* root system. Many insights into polarization and polar trafficking of proteins continue to be obtained by cellular analysis in the root tip. Recently, insights into the cytoskeletal machinery required for cell division reorientation in the root were obtained from a connection between developmentally regulated transcription factors and regulators of the microtubule cytoskeleton (Dhonukshe et al., 2012).

Perhaps the most exciting future direction resides in the broad evolutionary comparison of mechanisms discovered in roots to those operating in other contexts. When the root field noted long ago that the structure of the root could be considered the simplest postembryonic elaboration of the embryo axis, the notion arose that roots could reveal secrets relevant to all of plant development. Now, we know that all root patterning genes also pattern the embryo axis and we suspect that in many cases shoot development will use modified versions of the original networks. More broadly, the increasing depth of knowledge about root development might allow comparisons to regulatory systems logic used in all eukaryotes, such as histone modification and cell cycle/differentiation control, and inform their relationship to the so-called stem cell state. Now, my hope is that that we'll understand all this in another 20 years.

### Acknowledgements

Many thanks to Philip Benfey, Liam Dolan and John Schiefelbein for their comments on the manuscript and I am grateful for our enduring friendships.

### References

- Alfred, J. and Smith, J. (2008). Pointing a digit at digitised JEEM. *Development* **135**, 2339.
- Benfey, P. N., Linstead, P. J., Roberts, K., Schiefelbein, J. W., Hauser, M. T. and Aeschbacher, R. A. (1993). Root development in *Arabidopsis*: four mutants with dramatically altered root morphogenesis. *Development* **119**, 57-70.
- Birnbaum, K., Jung, J. W., Wang, J. Y., Lambert, G. M., Hirst, J. A., Galbraith, D. W. and Benfey, P. N. (2005). Cell type-specific expression profiling in plants via cell sorting of protoplasts from fluorescent reporter lines. *Nat. Methods* **2**, 615-619.
- Carlsbecker, A., Lee, J. Y., Roberts, C. J., Dettmer, J., Lehesranta, S., Zhou, J., Lindgren, O., Moreno-Risueno, M. A., Vatén, A., Thitamadee, S. et al. (2010). Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* **465**, 316-321.
- De Smet, I., Tetsumura, T., De Rybel, B., Frey, N. F., Laplaze, L., Casimiro, I., Swarup, R., Naudts, M., Vanneste, S., Audenaert, D. et al. (2007). Auxin-dependent regulation of lateral root positioning in the basal meristem of *Arabidopsis*. *Development* **134**, 681-690.
- Dello Ioio, R., Nakamura, K., Moubayidin, L., Perilli, S., Taniguchi, M., Morita, M. T., Aoyama, T., Costantino, P. and Sabatini, S. (2008). A genetic framework for the control of cell division and differentiation in the root meristem. *Science* **322**, 1380-1384.
- Dhonukshe, P., Weits, D. A., Cruz-Ramirez, A., Deinum, E. E., Tindemans, S. H., Kakar, K., Prasad, K., Mähönen, A. P., Ambrose, C., Sasabe, M. et al. (2012). A PLETHORA-auxin transcription module controls cell division plane rotation through MAP65 and CLASP. *Cell* **149**, 383-396.
- Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K. and Scheres, B. (1993). Cellular organisation of the *Arabidopsis thaliana* root. *Development* **119**, 71-84.
- Galinha, C., Hoffhuis, H., Luijten, M., Willemsen, V., Blilou, I., Heidstra, R. and Scheres, B. (2007). PLETHORA proteins as dose-dependent master regulators of *Arabidopsis* root development. *Nature* **449**, 1053-1057.
- Haseloff, J., Siemering, K. R., Prasher, D. C. and Hodge, S. (1997). Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic *Arabidopsis* plants brightly. *Proc. Natl. Acad. Sci. USA* **94**, 2122-2127.
- Helariutta, Y., Fukaki, H., Wysocka-Diller, J., Nakajima, K., Jung, J., Sena, G., Hauser, M. T. and Benfey, P. N. (2000). The SHORT-ROOT gene controls radial patterning of the *Arabidopsis* root through radial signaling. *Cell* **101**, 555-567.
- Kirik, V., Simon, M., Huelskamp, M. and Schiefelbein, J. (2004). The ENHANCER OF TRY AND CPC1 gene acts redundantly with TRIPTYCHON and CAPRICE in trichome and root hair cell patterning in *Arabidopsis*. *Dev. Biol.* **268**, 506-513.
- Laskowski, M., Grieneisen, V. A., Hoffhuis, H., Hove, C. A., Hogeweg, P., Marée, A. F. and Scheres, B. (2008). Root system architecture from coupling cell shape to auxin transport. *PLoS Biol.* **6**, e307.
- Lee, M. M. and Schiefelbein, J. (1999). WEREWOLF, a MYB-related protein in *Arabidopsis*, is a position-dependent regulator of epidermal cell patterning. *Cell* **99**, 473-483.
- Masucci, J. D. and Schiefelbein, J. W. (1996). Hormones act downstream of TTG and GL2 to promote root hair outgrowth during epidermis development in the *Arabidopsis* root. *Plant Cell* **8**, 1505-1517.
- Matsuzaki, Y., Ogawa-Ohnishi, M., Mori, A. and Matsubayashi, Y. (2010). Secreted peptide signals required for maintenance of root stem cell niche in *Arabidopsis*. *Science* **329**, 1065-1067.
- Mayer, K. F., Schoof, H., Haecker, A., Lenhard, M., Jürgens, G. and Laux, T. (1998). Role of WUSCHEL in regulating stem cell fate in the *Arabidopsis* shoot meristem. *Cell* **95**, 805-815.
- Menand, B., Yi, K., Jouannic, S., Hoffmann, L., Ryan, E., Linstead, P., Schaefer, D. G. and Dolan, L. (2007). An ancient mechanism controls the development of cells with a rooting function in land plants. *Science* **316**, 1477-1480.
- Moreno-Risueno, M. A., Van Norman, J. M., Moreno, A., Zhang, J., Ahnert, S. E. and Benfey, P. N. (2010). Oscillating gene expression determines competence for periodic *Arabidopsis* root branching. *Science* **329**, 1306-1311.
- Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J., Benfey, P., Leyser, O., Bechtold, N., Weisbeek, P. et al. (1999). An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. *Cell* **99**, 463-472.
- Sarkar, A. K., Luijten, M., Miyashima, S., Lenhard, M., Hashimoto, T., Nakajima, K., Scheres, B., Heidstra, R. and Laux, T. (2007). Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. *Nature* **446**, 811-814.
- Savage, N. S., Walker, T., Wieckowski, Y., Schiefelbein, J., Dolan, L. and Monk, N. A. (2008). A mutual support mechanism through intercellular movement of CAPRICE and GLABRA3 can pattern the *Arabidopsis* root epidermis. *PLoS Biol.* **6**, e235.
- Schiefelbein, J. W. and Benfey, P. N. (1991). The development of plant roots: new approaches to underground problems. *Plant Cell* **3**, 1147-1154.
- Tsukagoshi, H., Busch, W. and Benfey, P. N. (2010). Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. *Cell* **143**, 606-616.
- van den Berg, C., Willemsen, V., Hage, W., Weisbeek, P. and Scheres, B. (1995). Cell fate in the *Arabidopsis* root meristem determined by directional signalling. *Nature* **378**, 62-65.
- van den Berg, C., Willemsen, V., Hendriks, G., Weisbeek, P. and Scheres, B. (1997). Short-range control of cell differentiation in the *Arabidopsis* root meristem. *Nature* **390**, 287-289.
- Wysocka-Diller, J. W., Helariutta, Y., Fukaki, H., Malamy, J. E. and Benfey, P. N. (2000). Molecular analysis of SCARECROW function reveals a radial patterning mechanism common to root and shoot. *Development* **127**, 595-603.
- Xu, J., Hoffhuis, H., Heidstra, R., Sauer, M., Friml, J. and Scheres, B. (2006). A molecular framework for plant regeneration. *Science* **311**, 385-388.