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 Robarts’ 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...
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.

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**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; Matsuoka 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-Risueño et al., 2010).

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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.

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References


