

Root patterning: it takes two to tangle

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The mechanisms that pattern lateral root primordia are essential for the elaboration of root system architecture, a trait of key importance for future crop breeding. But which are most important: periodic or local cues? In this issue of *Journal of Experimental Botany* (pages 1411–1420), Kircher and Schopfer manipulate growth direction to demonstrate the importance of both sources of patterning information.

It may seem odd to use petri dishes to tease out the rules by which roots ramify underground – to aid the search for water and nutrients, circumnavigate soil particles and balance the forces on the aboveground plant for holdfast. But the simplicity of the ‘free running’ root-on-a-plate can reveal mechanisms of action that could easily go undetected when studying the system under more demanding conditions. Knowing how the basic mechanisms operate then helps us understand the underground plant system at a different level. This has led to precise descriptions of stages of lateral root primordium formation and outgrowth, the implication of many genes in this process, and to a recognition of the importance of overlying tissue in the emergence of lateral roots (see review by [Peret *et al.*, 2009](#)).

A particularly exciting question deals with lateral root patterning: where and when new lateral root primordia form. Together with the subsequent decision on how fast to grow out from these primordia, this process defines the basic features of root architecture. This question has been particularly puzzling because while there is some regularity to the spacing, the distance between individual lateral roots is not fixed. Indeed, it is far more variable than, say, the spacing of leaves about a stem.

Parallel lines of evidence

During the past decade two parallel lines of evidence have emerged from work on petri plates that emphasize either periodic or local cues for root primordium positioning ([Laskowski, 2013](#); [Van Norman *et al.*, 2013](#)). So, on the one hand there is evidence that cyclic activation of an auxin response reporter in elongating cells at the root tip marks these as founder cells for lateral root primordia ([Fig. 1A](#)) ([De Smet *et al.*, 2007](#); [Moreno-Risueno *et al.*, 2010](#); [Xuan](#)

[et al., 2015\). On the other hand, it has been shown that local curvature of roots matters for the selection of sites of lateral root primordium formation \(\[Fig. 1B\]\(#\)\) \(\[Ditengou *et al.*, 2008\]\(#\); \[Dubrovsky *et al.*, 2008\]\(#\); \[Laskowski *et al.*, 2008\]\(#\); \[Richter *et al.*, 2009\]\(#\)\).](#)

As frequently happens in science, particularly when interesting rival hypotheses emerge, both lines of evidence can be put diametrically against each other, holding that if either one of them turns out to be right, the other one must be wrong – a classic thesis–antithesis case. Cracks in that reasoning were already obvious from the published data. Neither theory for the origin of patterning information could easily explain all observations: local cues could not easily explain the correlation with oscillations (although the idea of spill-over from previous primordia was explored by [Laskowski *et al.*, 2008](#)); and global oscillations could not explain left–right patterning. Now [Kircher and Schopfer \(2016\)](#) provide the starting point for a synthesis of the existing ideas on patterning lateral root primordia. And the essentials of both ideas turn out to be correct.

Elegant experiments

The elegance of Kircher and Schopfer’s work lies in the deconvolution allowed by the experimental setup. A classic lateral root primordium stain was shown to match well with transgenic reporter dynamics, and hence used to map how often primordia arise and how rapidly they mature under a variety of ‘petri dish’ environments. Careful measurements with optimized growth conditions showed that the rate of primordium formation and their average frequency did not vary much when adjustments to the orientation of the agar plates modulated the direction of root growth. This allowed the authors to uncouple the effect of local curvature (which determined whether lateral root primordia formed on the left or right side of the main root) from the frequency of induction.

The key data from their paper (see [Fig. 1C](#)) should suffice to convince aficionados of either curvature or frequency cues that neither one of them is enough to determine the pattern of outgrowth. The frequency at which lateral roots grow out remains almost constant even in situations where the frequency of curvature changes. At the same time, the position

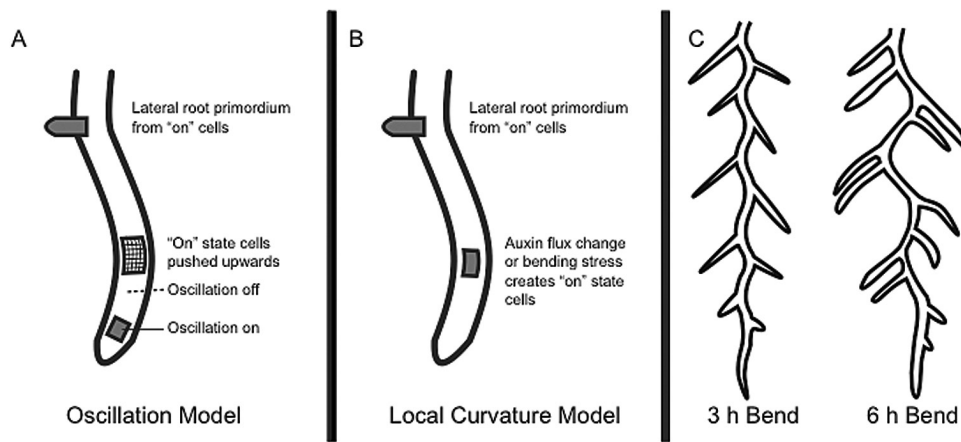


Fig. 1. Combining local and global cues for lateral root patterning. (A) The oscillation model posits an oscillating gene program in the basal meristem; cells which have the program 'on' will carry this state with them during expansion and are competent to form primordia. (B) The local cue model posits that cell shape and auxin flux changes and/or bending stress signals lead to the activation of the lateral root program. (C) The constant frequency but altered left-right positioning of lateral roots at different induced bending intervals reveals both frequency and bending cues.

of the lateral roots is fixed to the convex side of the curve and tends to be focused towards its apex. At some frequencies of curvature, this results in more than one lateral root emerging on the convex part of a single curve, relatively near to the point of peak curvature (Fig. 1C). Hence, the position of lateral roots is determined by local curvature while the overall frequency is independent of the frequency of curvature.

Why was this seemingly simple observation not made before? A part of the explanation is coincidence. The authors present convincing evidence that the normal waving growth rhythm of roots on near-vertical petri dishes, which arises from a combination of gravitropic and thigmotropic responses, happens to give a curvature frequency that maps within the 'one root per bend' domain. However, there are other factors involved. A previous paper where bending frequencies were modulated reported differences in the frequency of lateral root formation (Lucas *et al.*, 2008). These same authors also reported arrested primordia in their growth conditions, which is not observed in the studies by Kircher and Schopfer (2016). Hence, conditions of growth can play a role in these assays, as can be expected when one considers the potentially elaborate controls on lateral root outgrowth in complex environments. Without calling any growth condition 'better' or 'worse', it seems fair to make the point that if one can stabilize one aspect of pattern formation (here, frequency) and make the other vary, the claim that both must be (able to be) controlled independently is valid.

Synthesis and progress

The evidence presented here is an important step towards a synthesis. The proposal is that the variation in lateral root spacing may be explained by a combination of an oscillation of auxin response that establishes a region of competence, together with local cues including curvature that position primordia within that region. Curvature plays a role in such positioning, but other factors, such as hydrotaxis (Bao *et al.*, 2014), also matter, and discovering how these factors are integrated remains a challenge. Communication between pre-existing lateral root primordia (Laskowski *et al.*, 2008) and

events occurring in the oscillation zone may also explain part of the variation, subsequent to the first oscillation, and warrants further investigation.

The oscillation mechanism was discovered as a fluctuation in auxin response (De Smet *et al.*, 2007), and although not all auxin-responsive genes oscillate (Moreno-Risueno *et al.*, 2010), specific auxin sources in the root cap contribute to the oscillation (Xuan *et al.*, 2015). Subsequent to the oscillation, auxin and its transport are involved in many of the early steps of lateral root initiation that take place at the local site where a primordium is initiated on one or the other side of the axis (Benkova *et al.*, 2003; Okushima *et al.*, 2007; Dubrovsky *et al.*, 2008; De Rybel *et al.*, 2010). Even taking to heart the caveat that it is sometimes unwise to explain the unknown with the known, the re-occurrence of auxin in oscillatory priming and the initiation of lateral root primordium outgrowth means we have a good candidate for a synthesis of the two cues for lateral root patterning. After finding this out, the petri plates will have served their purpose and can be replaced by the underground challenges.

Key words: Arabidopsis, bending hypothesis, lateral root initiation, oscillation hypothesis, pattern formation, root architecture, waving growth.

Journal of Experimental Botany, Vol. 67 No. 5 pp. 1201-1203, 2016 doi: 10.1093/jxb/erw049

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Insight

Flavonoid biosynthesis and *Arabidopsis* genetics: more good music

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In this issue of *Journal of Experimental Botany* (pages 1505–1517), Ishihara *et al.* report the identification of a gene responsible for the production of flavonol 3-*O*-gentiobioside 7-*O*-rhamnosides by elegantly ticking the ivories of *Arabidopsis* genetics and genetic resources combined with straightforward metabolite analysis: it is a model case of functional evaluation.

Flavonoids are secondary metabolites derived from the phenylpropanoid biosynthetic pathway that occur in a huge number and variety in plants. They have been implicated in diverse processes, including pigmentation, redox and UV protection, plant–microbe interactions, development and regulation of auxin transport (Winkel-Shirley, 2006; Yonekura-Sakakibara *et al.*, 2008; Kuhn *et al.*, 2011; Peer *et al.*, 2011; Grunewald *et al.*, 2012; Buer *et al.*, 2013; Emiliani *et al.*, 2013; Saito *et al.*, 2013; Yin *et al.*, 2014; Ishihara *et al.*, 2016).

Genetics and flavonoid biosynthesis were already successfully engaged on the verge of *Arabidopsis* becoming the plant model organism. The *transparent testa* (*tt*) mutant loci, which affect the biosynthesis of flavonoids, defined easily scorable genetic markers due to the loss of seed coat pigmentation. Their molecular identification established many crucial steps in the biosynthesis of the flavonoid core structure (Koornneef *et al.*, 1983; Shirley *et al.*, 1995; Winkel-Shirley, 2006; Saito *et al.*, 2013). This core is formed by a phenyl ring condensed with an oxygen-containing heterocycle in different oxidation states which is further substituted at different positions with another phenyl side group. These variable cores constitute different flavonoid classes occurring

in plants and within a given plant species. However, only the decoration of these aglycones with various carbohydrate side chains and further chemical modification provides the full flavonoid range.

Again, genetics in combination with biochemistry, metabolite analyses and, in particular, gene co-expression patterns led to the identification of several UDP-carbohydrate-dependent glycosyltransferases (UGTs) conjugating flavonoids with different carbohydrates at different positions (Jones *et al.*, 2003; Yonekura-Sakakibara *et al.*, 2008, 2012). Nevertheless, there are still unresolved cases, one of them being the production of the *Arabidopsis* accession-specific flavonol 3-*O*-gentiobioside 7-*O*-rhamnosides (F3GG7Rs), comprising the flavonols kaempferol, quercetin or isorhamnetin with the specific carbohydrate decoration 3GG7R, which are most probably derived through glucosylation from flavonol 3-*O*-glucoside 7-*O*-rhamnoside (F3G7R) precursors.

A novel flavonol glucosyltransferase

Ishihara *et al.* (2016) have now identified a gene responsible for this final step in the production of F3GG7Rs. Previous reports that the accessions *Ler* and Nö-0, but not Col-0 and Cvi, contain F3GG7Rs were extended to a collection of 81 accessions, of which just half were F3GG7R-producers. The *Ler* F3GG7R trait was inherited in a dominant manner in a cross with the F3GG7R-lacking Col-0. Linkage analysis using an F3GG7R-metabotyped, 95-member-sized *Ler* × Col recombinant inbred (RI) population as