

VASCULAR DEVELOPMENT

Phloem research in full flow

In the phloem of vascular plants, distinct cell types are arranged in precise positions to ensure effective loading, transport and unloading of photosynthates and signalling molecules. Feedback between transcription factors and mobile peptides triggers and controls the differentiation of phloem at the growing root tip.

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The primary root of the model plant *Arabidopsis* has proved to be the go-to system for understanding the regulatory processes controlling phloem patterning. Two phloem poles form in *Arabidopsis* roots, and each phloem pole consists of two main cell types. The conductive cells are called sieve elements and can be divided into protosieve elements and metasieve elements, based on their position and the order in which they are specified. Companion cells maintain these sieve elements by assisting in loading and unloading of transported substances. Together these cell types are arranged in a cruciform shape, with each companion cell in contact with both sieve elements. This intricate geometry underpins the phloem function. Numerous genes critical to phloem development have been identified, but until now it was not clear how some of these key regulators interact to specify the spatial boundary for phloem. Writing in *Nature Plants*, Qian et al. report an elegant positive-feedback network between two groups of molecular components that defines strict domains of phloem specification and differentiation¹ (Fig. 1).

Recent studies identified a family of phloem-enriched DNA binding with one finger (DOF) transcription factors, also called PHLOEM EARLY DOFs (PEARs), that are required to promote cell division in and around the protosieve elements; these proteins also target central regulators of phloem development². Additionally, a number of genes have been shown to regulate phloem specification, including *BREVIS RADIX*³ and *OCTOPUS*⁴, which act in consort with a key peptide-receptor complex. In this pathway, three members of a large group of peptides (called CLEs; *CLAVATA3/EMBRYO SURROUNDING REGION-RELATED*) are perceived by BAM (BARELY ANY MERISTEM) and CIK (CLAVATA3 INSENSITIVE RECEPTOR KINASE) receptors/co-receptors and repress sieve element formation^{5,6}. Qian et al. now offer a molecular connection to link these

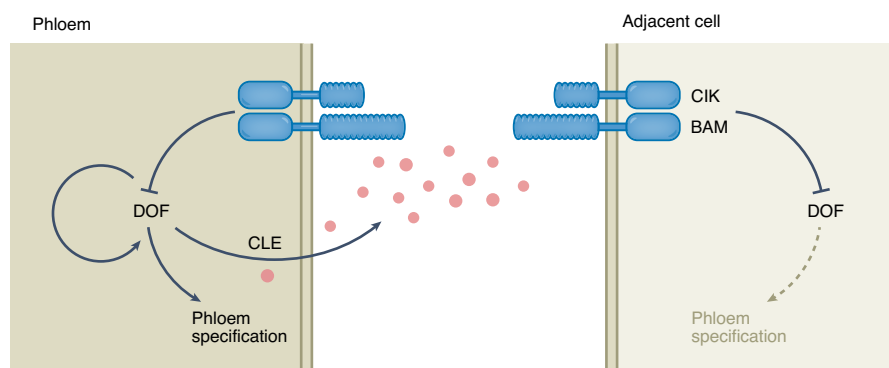


Fig. 1 | The *Arabidopsis* root phloem is a multicellular structure whose patterning and differentiation is controlled by lateral inhibition and positive feedback.

In the protophloem and metaphloem sieve elements and companion cells, the DOF transcription factors promote phloem specification. DOFs directly induce three CLE peptides, which move to neighbouring cells and, via BAM/CIK receptor kinases, stimulate the degradation of DOFs in the surrounding region. Meanwhile, DOFs promote their own expression, ensuring high DOF levels in phloem cells (dark green) and a lower level in adjacent cells (light green) and thus a strict tissue boundary.

networks. They show that not only do the DOF transcription factors control cell division, but that they are also necessary and sufficient for phloem specification. Crucially, the authors show that the DOFs are direct targets of CLEs, leading to the hypothesis that these peptides, in a signalling loop, could function downstream of DOFs as inhibitory signals preventing the specification of phloem in neighbouring cells.

To test this, the authors created a series of multiple mutants knocking out all three of the relevant CLEs. These mutants showed no effect on cell number, but plants displayed ectopic formation of both sieve elements and companion cells. The researchers then turned their attention to the components that perceive the CLE signals. They observed that both the BAM receptors and the CIK co-receptors were present in phloem and neighbouring cells, and that some of these components were ectopically expressed when DOFs were overexpressed, indicating that not only the peptides but also the receptors are induced by DOFs.

They observed ectopic phloem formation in both multiple *bam* mutants and multiple *cik* mutants, similar to that observed in the *cle* mutants. Together with an impressive array of genetic experiments, these data supported a model in which CLE expression is driven in the phloem downstream of DOFs. CLEs then diffuse outwards to surrounding cells where they bind to BAM and CIK receptors, activating downstream processes. This mechanistic loop is then closed as CLE activity leads to the degradation of DOF proteins, limiting their spatial reach. The team showed that as DOFs activate their own expression, this leads to a strong peak in DOF expression in protophloem, but to reduced levels in the surrounding cells due to repression via CLEs. Previous work has demonstrated the powerful effect these peptides have on phloem development and root growth in general, but this work reveals how lateral inhibition and a positive-feedback loop emerge from this network to produce control of the spatial domain of phloem specification.

In the early years of *Arabidopsis* research, the phloem remained an elusive and understudied part of the root, perhaps due to the inaccessibility and inherent complexity of the tissue. Within the last decade, phloem has gone from being one of the least well understood tissues in the root to arguably one of the best understood, with this study linking two of the major regulatory modules as an example of a significant advance. A curious aspect of this network is the high level of genetic redundancy. Indeed, sextuple mutants of *DOF* genes are required to reveal the full extent of their phenotype—an order of redundancy not reflected in analogous mechanisms such as that seen in

specification of ground tissue or root hair patterning^{7–9}.

The model of molecular interactions proposed by Qian et al. joins several dots in our understanding of phloem differentiation by linking two major pathways together. This work provides a clear mechanism of position dependent patterning in the phloem. These findings crystalize phloem specification as an exemplar model of spatial development. □

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Competing interests

The authors declare no competing interests.