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Current Opinion in Plant Biology

North, East, South, West: mapping vascular tissues onto the *Arabidopsis* root

John Vaughan-Hirsch, Benjamin Goodall and Anthony Bishopp

The *Arabidopsis* root has provided an excellent model for understanding patterning processes and cell fate specification. Vascular patterning represents an especially interesting process, as new positional information must be generated to transform an approximately radially symmetric root pole into a bisymmetric structure with a single xylem axis. This process requires both growth of the embryonic tissue alongside the subsequent patterning. Recently researchers have identified a series of transcription factors that modulate cell divisions to control vascular tissues growth. Spatial regulation in the signalling of two hormones, auxin and cytokinin, combine with other transcription factors to pattern the xylem axis. We are now witnessing the discovery of increasingly complex interactions between these hormones that can be interpreted through the use of mathematical models.

Address
Centre for Plant Integrative Biology and School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

Corresponding author: Bishopp, Anthony (anthony.bishopp@nottingham.ac.uk)

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Introduction

When one thinks of symmetry and patterning of organs, roots may not spring to mind as suitable organs to study three-dimensional patterns. However, the specification and differentiation of vascular tissues provides a symmetry-breaking event generating new positional information. In the model plant *Arabidopsis* this produces a bisymmetry in the primary root, with four poles located opposite each other, like the directions on a compass [1] (Figure 1). There are two xylem poles, each occupied by a single protoxylem cell, in the North and South positions. These poles are connected by a central axis containing metaxylem. Two phloem poles assume the East and West positions. The number and distribution of xylem and phloem are essential to maximize the transport of water, nutrients, photosynthetic assimilates and signals between organs to form an integrated network.

In this review, we investigate how genetic mechanisms combine with other sources of positional information to transform a vascular cylinder comprising just four vascular initial cells during embryogenesis, to a fully formed stele with about 45 cells containing a bisymmetric xylem axis. Two hormones, auxin and cytokinin, are instrumental in controlling this processes, but a suite of transcription factors and other molecules interact to regulate and fine-tune this process. We explore recent experimental and theoretical research that sheds light onto this symmetry breaking process. Although vascular patterning also involves the specification of phloem, and eventually secondary development they are beyond the scope of this article and we refer readers to other excellent recent reviews [2,3].

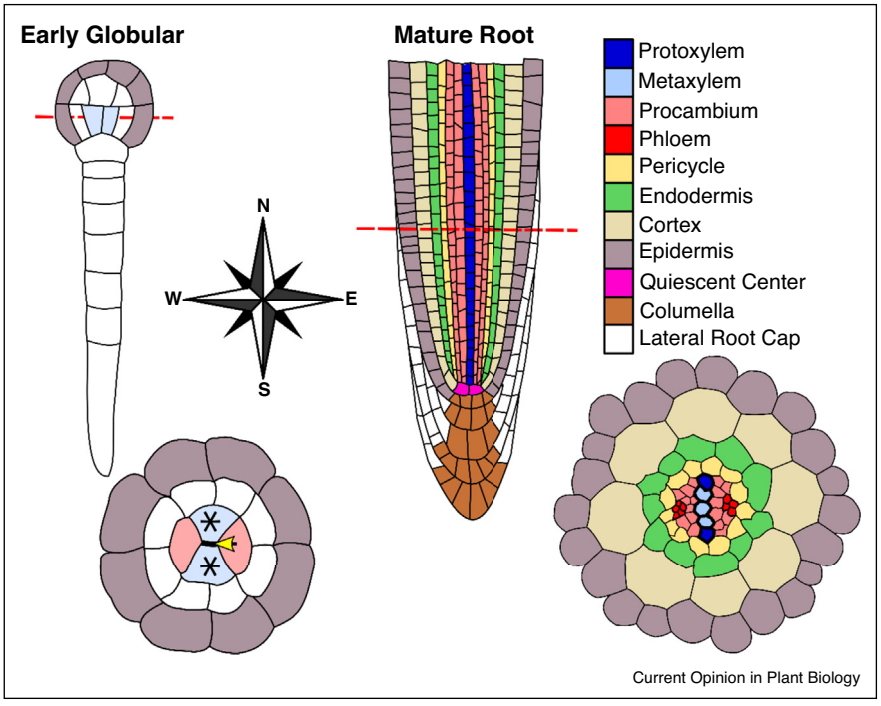
Specification of vascular cells

The precursor cells that will form the vascular cells are first present in the globular stage of the *Arabidopsis* embryo as four near-radially symmetric initial cells. In biology, the concept symmetry is always approximate and detailed analyses has revealed a cell-to-cell junction with the opposite cell exists for two of the four cells [4••]. All four of these cells undergo a series of periclinal cell divisions to generate both the ground and vascular tissues [1,5]. These periclinal cell divisions provide growth in the radial dimension.

Like many processes in plants [6], crosstalk between two hormones — auxin and cytokinin — take centre stage in regulating both the cell division establishing the vascular cylinder (stele) and the subsequent patterning of this tissue. The auxin signalling gene MONOPTEROS (MP)/AUXIN RESPONSE FACTOR 5 (ARF5) is central to this process, and *mp* mutants show strong defects in the formative divisions in provascular cells [7]. Perception of auxin through MP activates transcription of a set of downstream factors, including the basic helix-loop-helix protein TARGET OF MONOPTEROS 5 (TMO5) [8]. TMO5 forms a heterodimer with members of group of related proteins, the LONESOME HIGHWAY (LHW) family [9,10,11•]. These dimers regulate periclinal cell divisions through a localized increase in cytokinin biosynthesis caused through direct activation of the LONELY GUY 4 (LOG4) enzyme [4••,12••,13].

Manipulation of either *TMO5* or *LHW* levels has significant effects in regulating the size of the vascular cylinder.

Figure 1



The transition from the globular stage of embryogenesis to the mature root involves the proliferation of vascular cells and the establishment of a bisymmetric vascular pattern. In a cross section through the early globular embryo, four vascular cells are present. Two of these, in the North and South position (labelled *), are joined by a bridge (yellow arrow) and will receive an increased amount of auxin from the cotyledon apices. This bridge and the asymmetric input of auxin are essential to propagating the bisymmetric vascular pattern in the mature root with the xylem axis arranged on the same North-South plane [4••].

Multiple mutants with loss of function of either *tmo5* or *lhw* alongside their closest homologues have only a handful of vascular cells [9,10,11•]. Whilst, overexpressing both *TMO5* and *LHW* results in a massive increase in cell number throughout the root [11•]. If levels of the TMO5:LHW heterodimer have such an effect on cell division, how is the activity of TMO5:LHW regulated to precisely regulate cell number?

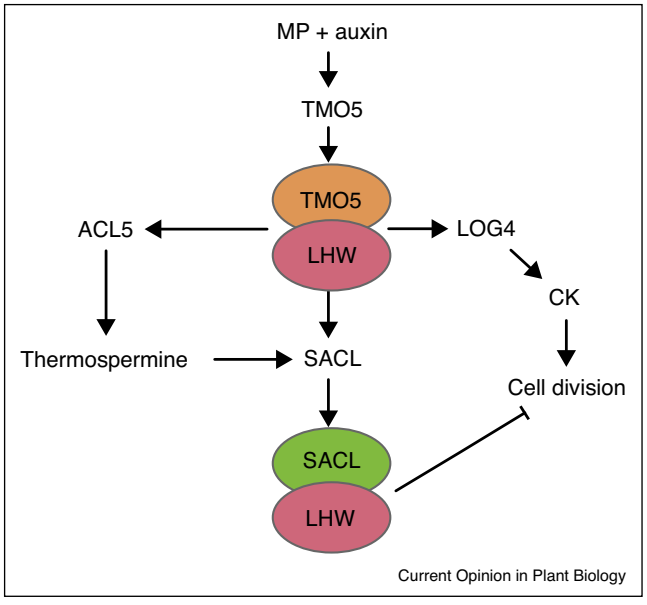
Thermospermine, a relatively new polyamine signalling molecule, has been shown to do exactly this [14••,15••]. A role for thermospermine had previously been implicated in vascular development as mutations in ACAULIS5 (ACL5) — a thermospermine biosynthesis gene [16] — results in dwarf plants with altered xylem patterning [17,18]. One key activity of thermospermine is that it promotes the accumulation of the SUPPRESSOR OF ACAULIS51-LIKE (SACL) family of basic helix-loop-helix proteins [19,20]. Members of this family can compete with TMO5 to dimerise with LHW and therefore restrict activity of the TMO5:LHW dimer. ACL5 and SACL3 have been identified as downstream targets of the TMO5:LHW dimer [15••] suggesting a feedback through which over proliferation of vascular cells can be prevented (Figure 2).

Establishing the xylem axis

Once the stele has been established, several interconnected networks are required to divide this space into discrete cell types, protoxylem, metaxylem, phloem and undifferentiated procambial cells. The earliest patterning event within the vascular cylinder is the specification of protoxylem identity, and a hormonal network containing many of the components described earlier controls this.

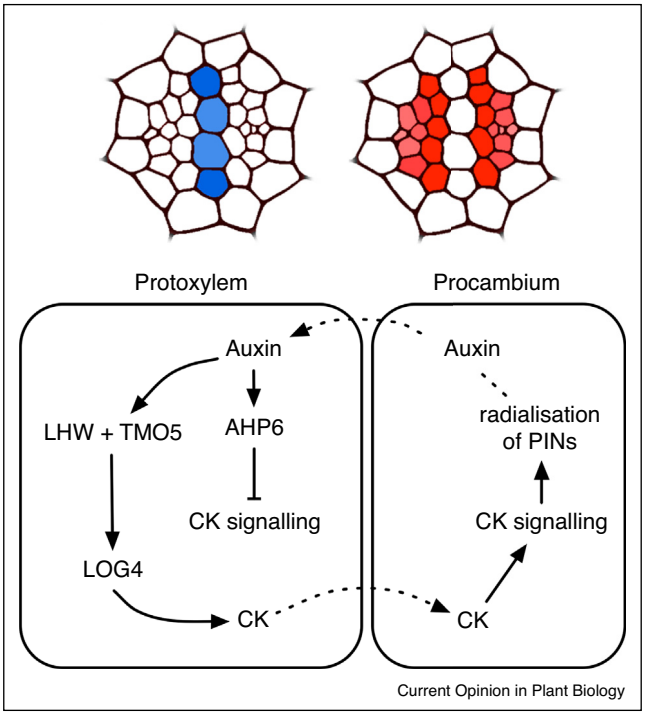
In this network, auxin and cytokinin signalling output occupy discrete domains and the antagonistic interaction between these hormones determines protoxylem versus procambial cell fate; auxin response is highest in the xylem axis, whilst cytokinin response is highest in adjacent cells (Figure 3a). Auxin signalling induces expression of, an inhibitor of cytokinin signalling, AHP6 at the marginal positions of this axis [21,22••], whilst cytokinin influences the expression and subcellular localisation of a sub set of auxin transporters known as PINs that redirect auxin towards the xylem axis [22••] (Figure 3b). Mathematical modelling approaches have provided conceptual verification that such a mechanism of mutual inhibition can generate distinct domains of hormonal output that

Figure 2



Schematic diagram showing how TMO5 and SACL compete to form heterodimers with LHW. In this figure TMO5, LHW and SACL represent multigenic groups of transcription factors rather than individual components.

Figure 3



Spatially specific domains of auxin and cytokinin response pattern the xylem axis. **(a)** The domain of high auxin response throughout the xylem axis is shown in the schematic in blue and high cytokinin signalling output shown in red. These outputs are similar to what can be seen with the DR5rev or TCSn marker [49,50]. AHP6 expression is restricted to the most marginal cells position of the xylem axis due to it being degraded in the centre by PHB [30••]. **(b)** These distinct domains of hormone signalling output are controlled by a mutual inhibition, through which auxin inhibits cytokinin response and cytokinin promotes the radial transport of auxin via PINs [22••]. In addition, auxin promotes TMO5, which dimerises with LHW to promote cytokinin production via LOG4 [4••,12••]. Cytokinin is then able to move to adjacent cells through diffusion.

can pattern the root [4••,23•,24,25]. However, one central question arises regarding how the asymmetry first arises.

Recent evidence suggests that an asymmetric input of auxin from the cotyledons acts as a signal to initiate symmetry breaking during embryogenesis, with cells with the highest auxin response going on to form the xylem axis. This asymmetry has been observed through auxin responsive markers migrating from the cotyledons to the root pole and driving higher expression in cells subtending the cotyledons [4••,22••]. Also, mutants with altered numbers of cotyledons show defects in vascular pattern in the hypocotyl (a tissue of embryonic origin) [26]. As LOG4 is a direct target of the TMO5:LHW complex, this asymmetry in auxin response also produces an asymmetry in LOG4 expression, with the highest levels in the same cells that have high auxin response [4••]. This might sound counter intuitive at first, but as the TMO5:LHW complex also promotes the expression of *AHP6* [4••,12••], it ensures that despite the presence of high cytokinin in these cells, they are non-responsive to cytokinin, maintaining protoxylem precursors in a non-dividing state [12••]. It has been hypothesised that cytokinin diffuses into the adjacent cells, establishing a maxima in the nearest neighbours promoting increased periclinal cell divisions in those cells flanking the axis [4••]. The actual mechanism and parameters have proved controversial [24], although the recent discovery of a PURINE PERMEASE as a cytokinin transporter [27] may provide new levels of regulation.

Whilst the role for the xylem as a non-responding source of cytokinin and the cotyledons as a source of auxin is clear during embryogenesis, this is less clear in the developing root. Cytokinin transported from the phloem is required to stabilise vascular pattern within growing roots [28]. However, a recent consensus between the vascular modelling papers concluded that cytokinin levels rather than asymmetries in cytokinin input was more important in patterning the root as stable vascular pattern could be achieved with a homogenous input of cytokinin [23•]. The link between growth and patterning plays a strong role, as mutants (such as *lhw*) with fewer vascular cells often produce just one xylem pole, overriding any initial pattern imposed by the cotyledons [9].

Other factors play an important role in regulating protoxylem versus procambial cell fate. The AT-HOOK MOTIF NUCLEAR LOCALIZED PROTEINS (AHL3 and AHL4) are expressed in the procambium and diffuse to all surrounding tissues to suppress protoxylem development [29]. It is unclear how they relate to the auxin-cytokinin network, although they appear to function independently of cytokinin signalling [29].

Specification of proto versus metaxylem cell fate

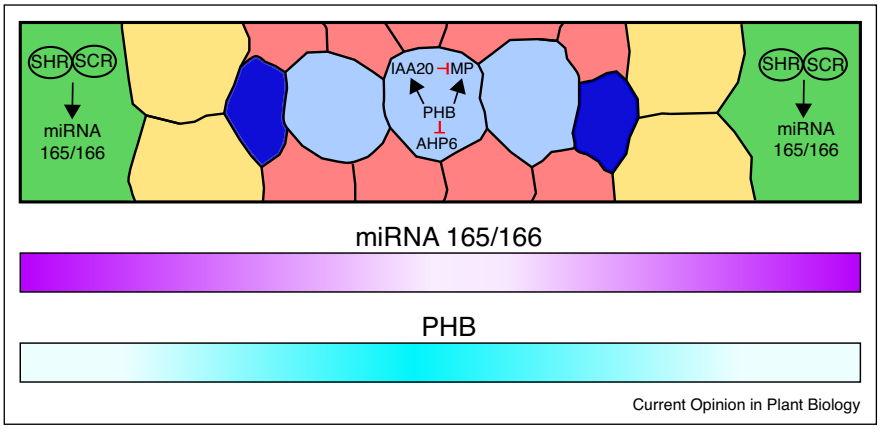
Whilst the auxin-cytokinin interaction and the function of AHL3/AHL4 control the protoxylem versus procambium cell fate, *PHABULOSA* (*PHB*) and four other closely related class III HD-ZIP transcription factors are required to determine proto versus metaxylem cell fate in a dose-dependent manner [30••,31•]. *PHB* levels are largely restricted to the central part of the root through miRNA165 and miRNA166; these diffuse from the surrounding tissue into the stele where they target the degradation of *PHB* mRNA [30••,31•]. The consequent gradient of HD-ZIPs drives the specification of xylem; cells with the highest levels of HD-ZIP form metaxylem and those with the lowest levels form protoxylem (Figure 4). PHB also restricts *AHP6* expression to the marginal positions within the xylem axis [30••] and feeds back on the auxin response by up-regulating both MP/ARF5 and its inhibitor IAA20 [32•]. Unlike most other AUX/IAAs, IAA20 and its homologue IAA30 are stable in the presence of auxin [33,34] and the double mutant *iaa20 iaa30* develops aberrant protoxylem, indicating the requirement for a certain level of inhibition of auxin response for normal vascular development. In addition, various auxin biosynthesis mutants display metaxylem defects which can be suppressed by increased expression of HD-ZIPs. This indicates that locally produced auxin in the root is required (in addition to polar transported auxin) for HD-ZIP expression and consequent metaxylem differentiation [35]. More recently, HD-ZIP IIIs and miRNA165/166 have been implemented in downstream processes through regulating the complex networks controlling secondary cell wall development [36,37].

Whilst these pathways control position in which cell fate is specified, a group of VASCULAR-RELATED NAC-DOMAIN PROTEINS (VNDs) are required for the terminal differentiation of xylem cells. Over-expression of VND6 or VND7 is sufficient to cause the trans-differentiation of diverse tissues into xylem vessels [38]. VND7 is regulated by another NAC-domain transcription factor, VNI2, which inhibits differentiation of xylem [39]. Recently, a wider network identified 14 transcription factors, which up regulate VNDs, integrating multiple developmental signals [40].

Other factors controlling xylem patterning

Other mutants have been identified displaying defects in xylem patterning, although these often have pleiotropic effects. It has recently been shown that N6-adenosine methylation of mRNA plays a role in root vascular patterning [41], and the translation elongation factor eIF5A has been shown to regulate protoxylem development through modulation of cytokinin signalling [42]. The polyamine spermidine (a precursor to thermospermine) is essential for activation of eIF5A by post-translational modification [43,44]. Changing patterns of gene

Figure 4



The activity of HD-ZIP III transcription factors determine protoxylem versus metaxylem cell identity. Protoxylem matures before surrounding tissues whereas metaxylem matures later, and these different tissue types have characteristic differences in secondary cell wall thickening. SHR is expressed in the stele and diffuses to the endodermis where it dimerises with SCR [51–54]. SHR-SCR induces expression of miRNA 165/166, which diffuse into the stele where they target *PHB* mRNA for degradation [30••,31•]. This leads to a gradient of *PHB* with the maxima in the centre. The collective action of PHB and the other HD-ZIP IIIs (PHV, REV, CNA and ATHB8) specify protoxylem or metaxylem in a dose dependent manner, with highest levels of HD-ZIP III activity promoting metaxylem, and lowest levels promoting protoxylem cell identity. PHB induces expression of *MP* and its inhibitor IAA20, providing a link with auxin signalling [32•].

expression via chromatin remodelling also affect vascular differentiation. Components of the Polycomb Repressive Complex 2 (PRC2), involved in histone methylation and consequent repressive chromatin formation, are expressed in a tissue-specific manner allowing differential gene regulation in non-vascular and vascular cells [45].

As well as feeding back on auxin signalling by supressing LHW:TMO5 function [14••,15••,20,46], thermospermine influences hormone signalling through Polyamine oxidase 5 (PAO5). This catalyses conversion of thermospermine to spermidine and is up regulated in the root vasculature by cytokinin and auxin treatment. Both mutant and over-expressing lines affect xylem patterning and control the expression of both hormonal response genes and vascular identity genes, such as *PHB*, *VND6* and *VND7* [47]. Biosynthesis of thermospermine is also regulated by class III HD-ZIPs, forming complex feedback loops to xylem development [48].

Perspectives for further study

Recent studies have provided a detailed molecular understanding concerning how four vascular initial cells can go on to form a bisymmetric pattern through establishing distinct domains of hormonal output. Whilst we have mathematical models that can explain the biological observations, the levels of feedback upon these hormones through components such as the AHL proteins and eIF5A have not been explored in this context. Whilst *Arabidopsis* has a bisymmetric vascular pattern with xylem poles at the North and South positions, other dicotyledonous plants such as *Medicago* and *Lotus* have roots with 3 or 4 vascular poles. It is tempting to hypothesise that like *Arabidopsis*, these species start with two xylem poles during embryogenesis and alternative patterns develop as the root pole grows. To test this requires early xylem marker lines in a number of different species. In this case a re-patterning event is needed to specify additional poles. *Arabidopsis* mutants with either smaller or larger vascular cylinders (e.g. *lhw*) with xylem one pole [9] or quadruple HD-ZIP mutants that have more vascular cells and occasionally produce a third pole [30••] suggest that this may be due differing spatial constraints.

Although the hormonal-mediated processes positioning the xylem axis are intimately linked with the HD-ZIP mediated processes patterning it, a clear molecular link has not yet been established with the VNDs that determine xylem identity. Current data suggest they are not direct targets of HD-ZIP/hormonal pathways [37,40], raising the possibility of a whole new group of intermediate factors yet to be discovered.

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