

Auxin signal transduction in Arabidopsis vein formation

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The plant signaling molecule auxin has been implicated in the control of a confounding multitude of diverse processes in plants, including leaf vascular patterning. In Arabidopsis leaves, expression of the *HD-ZIP III* gene *ATHB8* is initiated in files of isodiametric subepidermal cells that will elongate into vein-forming procambium. We have recently shown that *ATHB8* is transiently required for preprocambial development and procambium differentiation, and that permanence of the effects of loss of *ATHB8* function on vein formation depends on the activity of the auxin response factor MONOPTEROS (MP). Further, we have shown that the onset of *ATHB8* expression is directly and positively regulated by MP through an auxin-response element in the *ATHB8* promoter, suggesting a molecular path by which auxin signals are translated into vein patterning inputs. Within broad fields of MP expression, however, only a subset of cells initiates expression of *ATHB8*. Here we discuss putative mechanisms by which wide domains of MP expression could activate *ATHB8* transcription in single cell files.

the auxin efflux carrier PIN-FORMED1 (PIN1) are restricted to narrow fields that specify positions of procambial strand formation.^{6,8} Within wide PIN1 expression domains, expression of the class III *HOMEODOMAIN-LEUCINE ZIPPER* (*HD-ZIP III*) gene *ATHB8* is initiated in single files of leaf ground cells that will elongate into procambial cells.⁹⁻¹¹ Onset of *ATHB8* expression maintains its association with prospective procambial cells upon interference with vein patterning (reviewed in ref. 12), suggesting that inception of *ATHB8* expression identifies a ‘preprocambial’ cell state that preludes to vein formation.^{3,10,11}

Here we briefly discuss the role of *ATHB8* in leaf vascular development, the direct regulation of *ATHB8* preprocambial expression by the transcription factor MONOPTEROS/AUXIN RESPONSE FACTOR5 (MP/ARF5), and possible models by which broad domains of MP expression may lead to transcription of *ATHB8* in single cell files.

Functions of *ATHB8* in Leaf Vascular Development

Mature vein patterns in null *athb8* mutants do not conspicuously deviate from wild type;^{13,14} however, marker analysis in developing *athb8* leaves reveals transient abnormalities during early stages of leaf histogenesis,¹⁵ suggesting a role for *ATHB8* in preprocambial development and procambium differentiation (Fig. 1A). Such defects are corrected during *athb8* leaf development, suggesting either an ephemeral function of *ATHB8* in vein formation or functional redundancy among members of the *HD-ZIP III* gene family. Analysis of the genetic

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The plant vascular system is composed of bundles of cell files that extend throughout the plant, transporting water, minerals and nutrients.¹ Leaf vascular bundles (‘veins’) emerge de novo from a morphologically homogenous population of ground cells as strands of elongated, cytoplasm-dense procambial cells that generate mature vascular cells.² Accumulating evidence suggests that polar transport of the plant signaling molecule auxin plays a major role in vein patterning.³⁻⁸ In leaves, initially broad domains of expression of

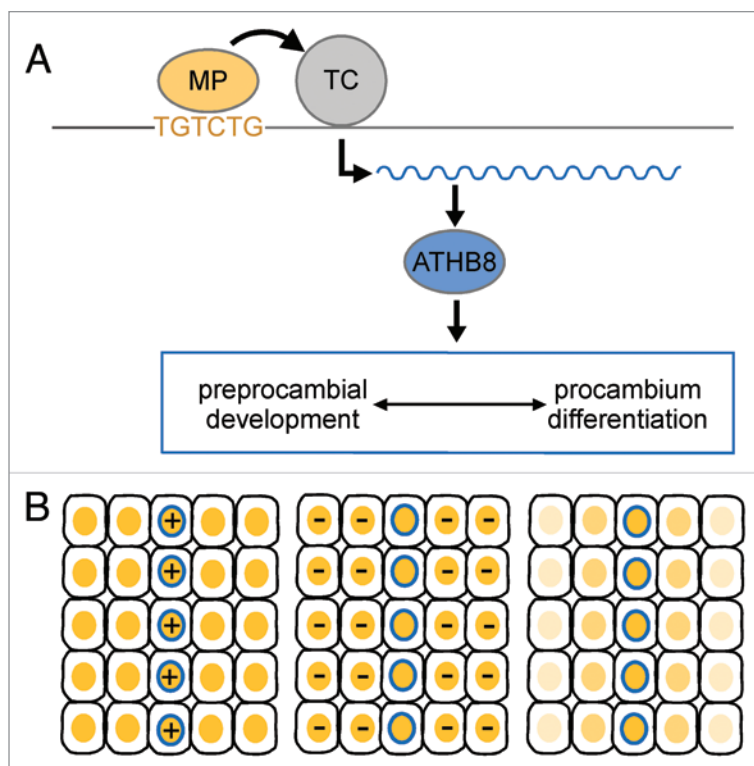


Figure 1. Summary and perspectives. (A) Molecular events in early vein formation. In Arabidopsis leaf primordia, MP binds a TGTCTG sequence motif in the *ATHB8* promoter and initiates *ATHB8* transcription (wavy line). *ATHB8*, in turn, controls preprocambial development and procambium differentiation. These two activities could represent completely separated and self-sufficient functions of *ATHB8* in leaf vascular development; they could overlap to various extents; or they could simply correspond to different readouts of the same regulatory potential. While the schematic nature of the relations presented here should be emphasized, the diagram illustrates how MP-dependent global auxin signaling inputs are molecularly partitioned into defined patterning events in leaf vascular development. TC, transcription complex. (B) Mechanisms for restriction of *ATHB8* transcription to a subpopulation of MP-expressing cells. At least three formally different scenarios can be envisaged. According to the first (left), expression of *ATHB8* (ring) in a subset of the cells expressing MP (circle) would depend on the presence of an activating signal (plus sign) in preprocambial cells. This cue may not depend on the transcriptional regulatory properties of MP and does not necessarily implicate sequence-specific DNA binding activities, but could reflect, for example, different chromatin configurations at the *ATHB8* promoter in different cells.^{24,25} In the second scenario (central), confined activation of *ATHB8* expression within wide fields of MP expression would rely upon removal of an inhibiting signal (minus sign) in preprocambial cells, such as transcription-independent degradation of repressors of the Aux/IAA family.²³ Finally, according to the third scenario, MP protein would be present in an instructive gradient in leaf primordia with maximum expression in cells that initiate *ATHB8* transcription. Because the *ATHB8* preprocambial element is expected to be suboptimal for MP binding,²⁶ activation of *ATHB8* expression would only occur where MP levels are sufficiently high.

interaction between mutations in *ATHB8* and in *MP*—a direct upstream regulator of *ATHB8*¹⁵—provides insight into this matter. Absence of *ATHB8* activity results in a small increase in the sensitivity of leaves to auxin transport inhibition; however, in a weak *mp* background, additional mutation of *ATHB8* enhances the phenotypic severity of both untreated and auxin transport-inhibited leaves. This observation suggests that loss of *ATHB8* function

has long-lasting consequences on vein formation, but that the role of *ATHB8* in this process is masked in the *athb8* mutant by wild-type *MP* activity. The downregulation of expression of other members of the *HD-ZIP III* family in *mp*³ suggests that resolution of early vascular defects in *athb8* leaves may depend on MP-regulated recruitment of *HD-ZIP III* gene redundant activities. All members of the *HD-ZIP III* family have been suggested to have roles in

vascular development.¹⁴ However, unlike *ATHB8*, the other members have additionally been implicated in regulation of acquisition of leaf adaxial-abaxial polarity: a process that has repercussions on leaf morphogenesis and vein patterning.¹⁶ Therefore, understanding nonredundant and overlapping functions of *HD-ZIP III* genes in leaf vascular development will require careful separation of their specific roles in vein formation from vascular readouts of their activities in leaf adaxial-abaxial patterning.

Regulation of *ATHB8* Preprocambial Expression

Both auxin responsiveness and correct initiation of *ATHB8* expression depend on a TGTCTG element in the *ATHB8* promoter¹⁵ (Fig. 1A). This motif closely resembles the canonical auxin response element TGTCTC necessary for auxin inducible gene expression.^{17,18} The TGTCTG sequence in the *ATHB8* promoter is an *in vivo* binding site for MP/ARF5¹⁵ (Fig. 1A), and onset of *ATHB8* expression is extremely delayed in a strong *mp* background.¹⁵ In addition to their reduced organ vascularization, *mp* mutants are characterized by severe auxin insensitivity and defects both in the formation of the apical-basal axis of the embryo and in the patterned outgrowth of post-embryonic lateral organs, suggesting a broader role for *MP* in auxin-mediated axial patterning.^{3,19–22} MP/ARF5 belongs to the Auxin Response Factor (ARF) family of transcription factors and is one of five ARFs identified as transcriptional activators (reviewed in ref. 23). The five activating ARFs are characterized by a high degree of functional redundancy, raising the possibility that any of them could regulate transcription of *ATHB8*. However, the inability of a null *athb8* mutation to enhance vascular patterning defects in mature leaves of a strong *mp* mutant¹⁵ suggests that nonredundant functions of *ATHB8* in vein formation are primarily dependent on *MP*, and that the contribution of other ARFs to regulation of *ATHB8* activity in vein ontogeny is probably minor.

Like other members of the *HD-ZIP III* family, *ATHB8* undergoes post-transcriptional regulation by

microRNA activity.¹⁶ However, this level of control does not seem to affect initiation of *ATHB8* expression in leaves,¹⁵ suggesting that *ATHB8* preprocambial expression is mainly dependent on MP activity.

Stage-Specific Expression Dynamics in Early Vein Development

Similar to PIN1, MP expression is initially detected in broad domains that eventually narrow to sites of vein formation,^{8,20} while *ATHB8* expression is initiated in single files of isodiametric ground cells within wide MP expression domains.¹⁵ As the TGTCTG element in the *ATHB8* promoter is a direct target of MP activity, why is *ATHB8* transcription not promoted in all MP-expressing cells? At least three formally different possibilities are conceivable (Fig. 1B). The first is that MP is not sufficient to activate *ATHB8* transcription and requires the simultaneous presence of a preprocambial-specific co-activating signal. The second is that MP activity depends on the specific removal of an inhibiting cue in preprocambial cells. Finally, the third is that MP protein is present in an instructive gradient in leaf primordia, with maximal expression in cells that will activate *ATHB8* transcription. It should be emphasized that these scenarios do not necessarily exclude one another, but that they could all, to varying extents, co-exist. In the future, it will be interesting to understand how *ATHB8* expression is initiated in single cell files within broad domains of MP expression. Nevertheless, identifying a role for *ATHB8* in vein formation and its direct regulation by MP

provides a molecular mechanism through which general auxin signal transduction controls leaf vascular development.

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