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A dominant mutation reveals asymmetry in *MP*/*ARF5* function along the adaxial-abaxial axis of shoot lateral organs

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Abbreviations: ARF, Auxin Response Factor; *MP*, *MONOPTEROS*; HD-ZIPIII, class III homeodomain-leucine zipper; KAN, KANADI; PIN1, PIN-FORMED1; ETT, ETTIN; GFP, green fluorescent protein; GUS, β-glucuronidase

The establishment of adaxial-abaxial polarity in plant lateral organs involves elaborate interactions between members of several transcription factor families, including the Auxin Response Factors (ARFs). We previously described a dominant allele of *ARF5*/*MONOPTEROS* (*MP*), termed *MP*Δ, which causes severe vascular hypertrophy in shoot lateral organs. Here we report that these organs are also disrupted in adaxial-abaxial polarity. Other *MP*Δ lateral organs with decreased vasculature show similar disruptions, suggesting that *MP* impinges on organ polarity through pathways separate from its role in promoting vascularization. Furthermore, we demonstrate that *MP*Δ exhibits an adaxial-abaxial asymmetry in its ability to influence organ development. Since ARFs previously implicated in polarity establishment function as transcriptional repressors, the transcriptional activator MP represents a novel link between auxin signal transduction and adaxial-abaxial polarity.

Shoot lateral organs acquire a polarity axis based on proximity to the apical meristem from which they originate. The adaxial domain develops adjacent to the meristem and will differentiate into the upper portion of the organ, while the distant abaxial domain will give rise to the lower side of the organ. Class III homeodomain-leucine zipper (HD-ZIPIII) genes are important determinants of shoot meristem function and adaxial fate, while members of the KANADI (KAN) family of GARP transcription factors specify abaxial fate.[1] The juxtaposition of adaxial and abaxial fate appears to be required for laminar outgrowth of lateral organs.[2]

A number of observations have implicated the phytohormone auxin in the formation of the adaxial-abaxial axis. First, routes of auxin transport, defined by the distribution and subcellular localization of the auxin efflux facilitator PIN-FORMED1 (PIN1), delineate the adaxial-abaxial boundary of lateral primordia and may therefore serve as positional reference points for polarity establishment.[3] Additionally, auxin induces the expression of many HD-ZIPIII genes.[4,5] Finally, the Auxin Response Factors (ARFs) ARF3/ETTIN (ETT) and ARF4 cooperatively promote abaxial identity in lateral organs.[6]

We have recently described the developmental effects of expressing truncated alleles of *ARF5*/*MONOPTEROS* (*MP*) in Arabidopsis.[7] The proteins encoded by these deletion alleles (termed *MP*Δ) lack dimerization domains III and IV and are therefore predicted to escape the negative regulation conferred by repressive Aux/IAA binding partners. The lateral organs of *MP*Δ plants exhibit vascular hypertrophy and restricted expansion of the lamina. Here we report other phenotypic defects of *MP*Δ that are consistent with adaxial-abaxial disruption and speculate on the link between MP function and the establishment of lateral organ polarity.

*MP*Δ shoot lateral organs exhibit a narrow morphology due to the lack of laminar expansion (**Fig. 1**).[7] In leaves, this is invariably associated with a striking increase in vascular production due to ectopic expression of the procambium-selecting gene *PIN1*.[7] As a result, an increased number of ground meristem cells are recruited into the vascular lineage, leaving a vastly reduced pool of cells capable of growth in the lateral axis.

In addition to this reduction in laminar growth, *MP*Δ leaves can be cup-shaped in appearance (**Fig. 1C and D**). This trait is produced in lateral organs when the proper juxtaposition of adaxial and abaxial fate is unbalanced due to mutation and/or gene misregulation.[8-10] Therefore, these observations suggest that MP, like other ARFs including ETT and ARF4, influences organ polarity.

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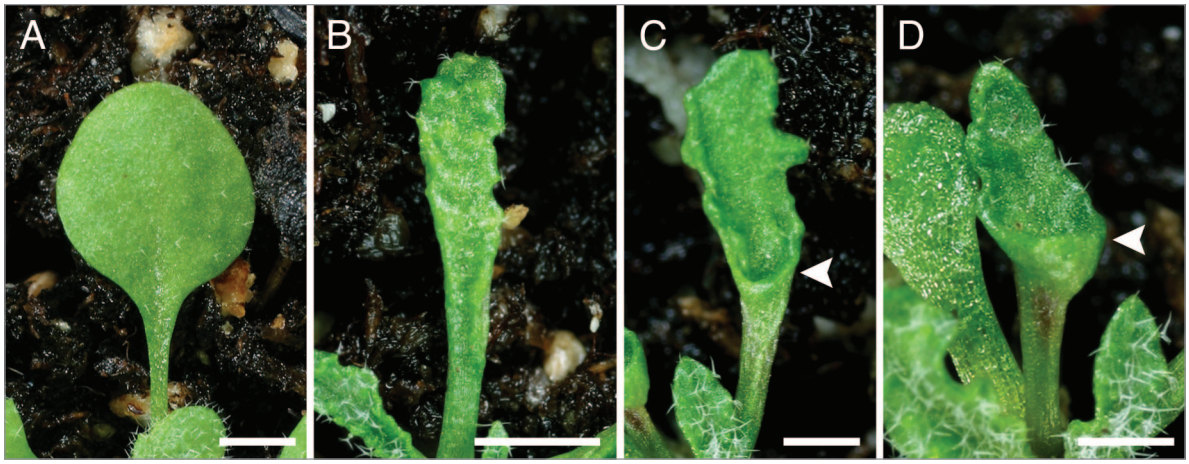

Figure 1. *MP*Δ disrupts leaf adaxial-abaxial polarity. (A) Wild-type first leaf. (B) *MP*Δ first leaf lacking an expanded lamina. (C and D) *MP*Δ-*3* rosette leaves displaying cup/trumpet-shape (arrowheads). Bars: (A and B) 2 mm; (C and D) 1 mm.

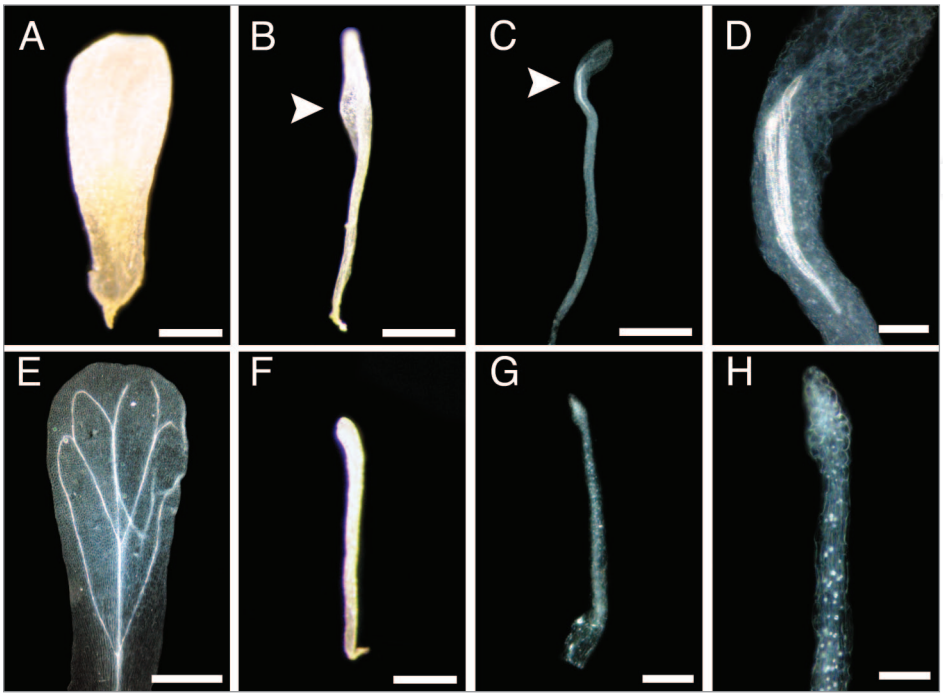

Figure 2. Radialized *MP*Δ floral organs are defective in vascular production. (A) Wild-type petal. (B) *MP*Δ petal with laminar expansion at its distal end (arrowhead). (C and D) Cleared petal shown in (B) with vascular production (xylem) restricted to laterally expanded region (arrowhead). (E) Cleared wild-type petal with looping vasculature. (F) Radialized *MP*Δ floral organ. (G) Cleared radialized *MP*Δ floral organ devoid of vasculature. (H) Close view of organ depicted in (G). (C–E, G and H) Dark field images. Bars: (A–C, E and F) 0.5 mm; (G) 0.2 mm; (D and H) 0.05 mm.

Because organ and vascular development are closely linked processes,[11] the impingement of *MP*Δ on organ polarity may be an indirect effect of vascular hypertrophy. However, aspects of *MP*Δ floral organ development suggest that this is not the case. For instance, while most *MP*Δ floral organs are narrow and have increased vascularization,[7] some display varying degrees of radial symmetry and the absence of vasculature altogether (**Fig. 2**). These characteristics frequently result from disrupted organ polarity.[8,12-14]

Like many transcriptional regulators involved in the establishment of polarity, MP is asymmetrically distributed in young leaf primordia, showing strong adaxial expression (**Fig. 3A**).[7] We sought to determine whether the effect of MPΔ on vascular production and organ polarity was unique to this adaxial expression domain. We therefore placed *MP*Δ under the control of the *UAS* cis-regulatory sequence and introduced this transgene into trans-acting GAL4-GFP enhancer trap lines to drive expression in adaxial or abaxial organ domains. While the *UAS::MP*Δ transgene on its own had no effect on leaf development (**Fig. 3B**), driving its expression in an adaxial/proximal domain of leaf primordia (similar to that of endogenous *MP*) resulted in a marked increase in vascular production (**Fig. 3C–E**). While cup-shaped leaves were not observed in this background, ectopic lobed outgrowths of the lamina, which have been linked to adaxial-abaxial polarity disruption,[15,16] were frequent (**Fig. 3E**). In contrast, driving expression of *UAS::MP*Δ in the abaxial region of leaf primordia was insufficient to increase vascularization or significantly affect organ morphology (**Fig. 3F–H**). These results indicate that the effect of *MP*Δ on lateral organ development depends on its adaxial expression and suggests qualitative differences between leaf polarity domains regarding competence to respond to *MP* function.

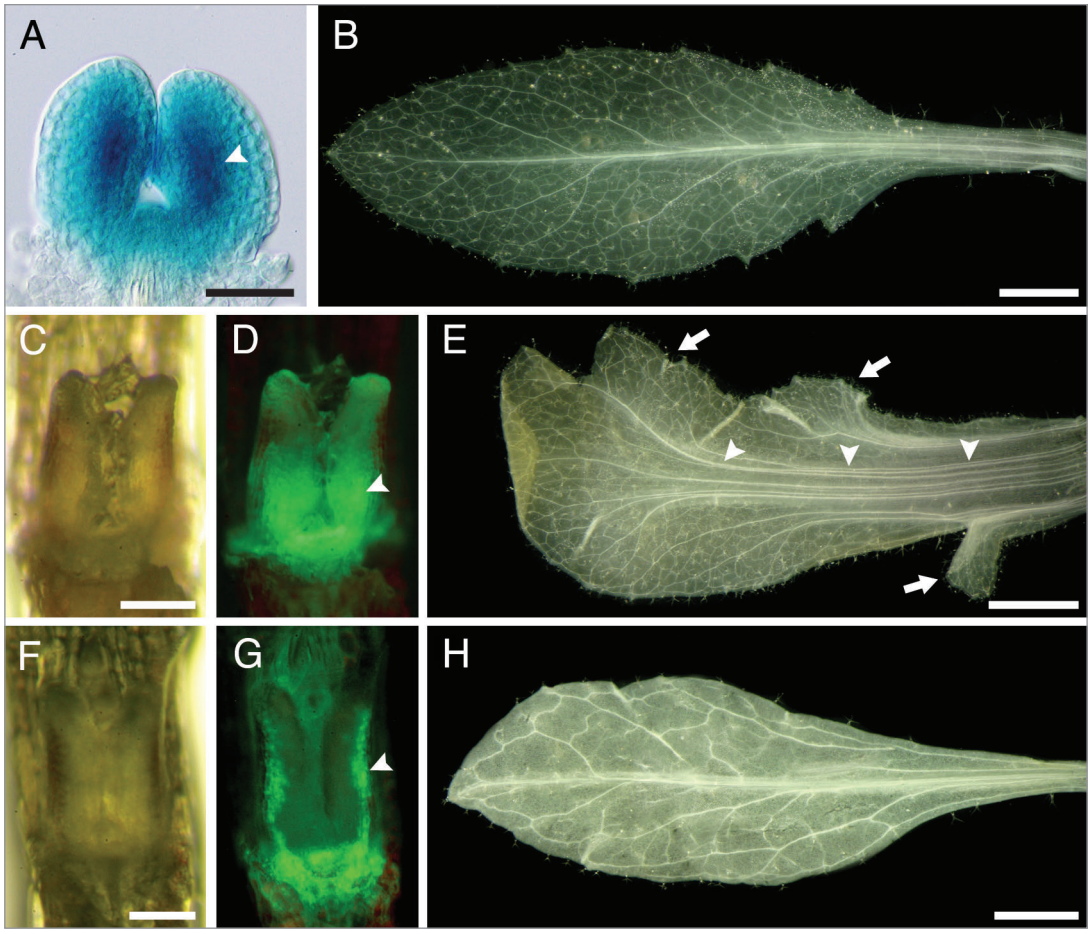

Figure 3. *MP* expression and *MP*Δ function show an adaxial bias in leaf development. (A) *MP::MP:GUS* reporter expression in leaf primordia, with stronger signal in the adaxial/proximal region (arrowhead). (B) Cleared *UAS::MP*Δ leaf with a normal reticulate venation pattern. (C) Bright field and (D) GFP fluorescence images of leaf primordia of GAL4-GFP enhancer trap line CS70055. Arrowhead in (D) indicates GFP expression in adaxial/proximal leaf domain. (E) *UAS::MP*Δ; CS70055 cleared leaf exhibiting vascular hypertrophy (arrowheads) and ectopic lobing of the lamina (arrows). (F) Bright field and (G) GFP fluorescence images of leaf primordia of GAL4-GFP enhancer trap line CS70128. Arrowhead in (G) shows GFP expression in the abaxial leaf domain. (H) *UAS::MP*Δ; CS70128 cleared leaf showing a lack of vascular enhancement. (B, E and H) Dark field images. Bars: (A) 0.05 mm; (B, E and H) 2 mm; (C and F) 0.1 mm.

Based on our prior investigation of *MP*Δ, we proposed that the expression of ARF derivatives lacking dimerization domains III and IV could serve as novel genetic tools to interrogate ARF function.[7] This idea is extended by the present work which implicates *MP* in adaxial-abaxial polarity establishment, an association not revealed by previous *MP* loss- or gain-of-function studies. Other experimental observations have hinted at such a relationship, including the regulation of HD-ZIPIII gene family members by MP[17,18] and the involvement of other ARFs in polarity formation.[6]

The disruption of polarity in *MP*Δ leaves may be a secondary consequence of vast increases in vascularization, as these two developmental processes are intimately linked.[11] HD-ZIPIII genes, for example, are involved in both vascular patterning and adaxial fate specification, and their increased expression domains in *MP*Δ leaves may impinge upon the balance between adaxial and abaxial fate. The abaxial-promoting *KAN* genes may also be affected by *MP*Δ vascular hypertrophy. *KAN1* negatively affects the expression and function of PIN1.[19,20] The modulation of many auxin-related processes involves feedback mechanisms, and an environment dominated by PIN1 activity may similarly feedback upon *KAN* function.[19] Furthermore, positions of PIN1 expression have been proposed to serve as reference points for lateral organ adaxial-abaxial boundaries;[3] therefore, the widening of PIN1 expression domains in *MP*Δ would be expected to significantly alter organ polarity.

Other observations argue against the idea that MPΔ influences organ polarity solely by increasing vascularization. For instance, *MP*Δ floral organs can exhibit severe disruptions in their polarity (such as radialization) while producing little to no vasculature. This would suggest that MPΔ influences polarity in a direct fashion independent of its role in promoting the formation of vasculature.

MPΔ affected leaf development only when expressed in the adaxial domain of the organ. This indicates that the abaxial region of the leaf is unable to respond to MPΔ activity, and this qualitative difference between primordia domains may contribute to the effect of MPΔ on organ polarity. It is possible that cofactors required for MP activity are confined to the adaxial

domain of lateral organs. Alternatively, the activity of repressor ARFs such as ETT and ARF4 in the abaxial domain may compete with MPΔ (an activating ARF) for protein co-factors and/or DNA binding sites, thereby negating its effects.

While our results add MP to the growing list of transcriptional regulators implicated in lateral organ polarity establishment, future experiments are needed to better define its role in this process. This includes detailed analysis of the internal anatomy of affected *MP*Δ organs, such as the orientation of phloem and xylem tissue in vascular bundles (where present). Additionally, genetic interactions between *mp* loss-of-function alleles and classical adaxial-abaxial polarity mutants could also prove informative. Such investigations will serve to further our understanding of the role of auxin signal transduction in lateral organ development.

Materials and Methods

Plant growth conditions, transformation procedures and the production of *MP*Δ and *MP::MP:GUS* transgenic lines have been previously described in reference 7. To generate transgene *UAS::MP*Δ, the coding sequence of *MP*Δ was amplified by PCR, placed under the control of the 6x*UAS* cis-regulatory sequence, and introduced into the binary vector pGPTV-KAN.[21] GAL4-GFP enhancer trap lines[22] were obtained from the Arabidopsis Biological Resource Center (www.arabidopsis.org) (donated by S. Poethig). Microscopy, including capture of dark-field images, was performed as previously reported in reference 7.

Disclosure of Potential Conflicts of Interest

The authors declare no conflicts of interest, financial or otherwise.

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