Multiple *MONOPTEROS*-Dependent Pathways Are Involved in Leaf Initiation[1][C][W][OA]

Mathias Schuetz, Thomas Berleth, and Jim Mattsson*

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6 (M.S., J.M.); and Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada M5S 1A1 (T.B.)

Initiation of leaves at the flanks of the shoot apical meristem occurs at sites of auxin accumulation and pronounced expression of auxin-inducible *PIN-FORMED1* (*PIN*) genes, suggesting a feedback loop to progressively focus auxin in concrete spots. Because *PIN* expression is regulated by auxin response factor activity, including *MONOPTEROS* (*MP*), it appeared possible that *MP* affects leaf formation as a positive regulator of *PIN* genes and auxin transport. Here, we analyze a novel, completely leafless phenotype arising from simultaneous interference with both auxin signaling and auxin transport. We show that *mp pin1* double mutants, as well as *mp* mutants treated with auxin-efflux inhibitors, display synergistic abnormalities not seen in wild type regardless of how strongly auxin transport was reduced. The synergism of abnormalities indicates that the role of *MP* in shoot meristem organization is not limited to auxin transport regulation. In the *mp* mutant background, auxin transport inhibition completely abolishes leaf formation. Instead of forming leaves, the abnormal shoot meristems dramatically increase in size, harboring correspondingly enlarged expression domains of *CLAVATA3* and *SHOOTMERISTEMLESS*, molecular markers for the central stem cell zone and the complete meristem, respectively. The observed synergism under conditions of auxin efflux inhibition was further supported by an unrestricted *PIN1* expression in *mp* meristems, as compared to a partial restriction in wild-type meristems. Auxin transport-inhibited *mp* meristems also lacked detectable auxin maxima. We conclude that *MP* promotes the focusing of auxin and leaf initiation in part through pathways not affected by auxin efflux inhibitors.

Plants continuously produce lateral organs, primarily leaves and flowers, at the flanks of shoot apical meristems (SAMs). Considerable advances have been made over the past 10 years on the understanding of the genetic basis of meristem maintenance, proliferation, and lateral organ formation (for review, see Williams and Fletcher, 2005; Carraro et al., 2006; Shani et al., 2006; Tucker and Laux, 2007). At the center of the meristem, a central zone (CZ) of generally less frequently dividing cells provides cells for the more frequently dividing surrounding peripheral zone (PZ) and underlying rib zone (RZ; Reddy et al., 2004). Together, the CZ, PZ, and RZ make up the SAM. The meristem provides cells for lateral organ (i.e. leaf and flower formation) and underlying pith formation. The size of the CZ is regulated by a feedback loop involving the *CLAVATA* (*CLV*) genes and the *WUSCHEL* (*WUS*) gene (Fletcher et al., 1999; Schoof et al., 2000; Clark, 2001). The meristem is specified and maintained by the *SHOOTMERISTEMLESS* (*STM*) gene (Long et al., 1996; Muday and DeLong, 2001; Kumaran et al., 2002), along with other members of the same gene family, primarily *BREVIPEDICELLUS* (*BP*)/*KNAT1* and *KNAT2* (Chuck et al., 1996; Ori et al., 2000; Muday and DeLong, 2001; Byrne et al., 2002). *STM* appears to carry out this function at least in part by preventing the expression of *ASYMMETRIC LEAVES1* (*AS1*) in the meristem (Byrne et al., 2000; Long and Barton, 2000). *AS1*, in turn, promotes lateral organ formation at the flanks of the PZ by downregulating *BP*/*KNAT1* and *KNAT2* at the sites of lateral organ formation (Byrne et al., 2000, 2002). Lateral organ formation also depends on the *AINTEGUMENTA* (*ANT*) gene, which promotes cell proliferation in these structures (Mizukami and Fischer, 2000). Both *ANT* and *AS1* have been used as early molecular markers for the formation of lateral organs (Long and Barton, 1998; Byrne et al., 2000; Vernoux et al., 2000). Although some of the interactions of meristem-organizing genes have been documented, clear evidence of how primordia-specific genes become expressed at the sites of lateral organ formation remain elusive. The separation of the emerging lateral organs is promoted by several genes, most notably the *CUP-SHAPED COTYLEDON* genes, adding another level of regulation involved in lateral organ formation (Aida et al., 1997, 1999; Hibara et al., 2003; Vroemen et al., 2003; Koyama et al., 2007).

[1] This work was supported by Natural Sciences and Engineering Research Council (NSERC) discovery grants (to J.M. and T.B.) and an NSERC graduate student fellowship (to M.S.).

* Corresponding author; e-mail mattsson@sfu.ca.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Jim Mattsson (mattsson@sfu.ca).

[C] Some figures in this article are displayed in color online but in black and white in the print edition.

[W] The online version of this article contains Web-only data.

[OA] Open Access articles can be viewed online without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.108.119396

It has long been known that the formation of lateral organs can be influenced by the plant hormone auxin (Reinhardt et al., 2000, and refs. therein). Application of auxin, as well as auxin efflux inhibitors, results in a range of phenotypes from altered numbers and positions of flowers and leaves to a complete block of flower formation from reproductive SAMs (Wardlaw, 1949; Meicenheimer, 1981; Okada et al., 1991; Mattsson et al., 1999). Recent advances suggest that auxin accumulation is required for lateral organ initiation and that auxin is transported to these sites by membrane-bound efflux transport proteins that polarly localize to apical or basal ends of cells (Benkova et al., 2003; Reinhardt et al., 2003; Friml et al., 2004; Heisler et al., 2005; Petrasek et al., 2006). A key component in this process is *PIN-FORMED1* (*PIN1*), a member of the PIN family of membrane-bound auxin efflux proteins (Okada et al., 1991; Galweiler et al., 1998). Loss-of-function mutations in the *PIN1* gene result in reduced auxin transport and defective cotyledon and flower formation (Okada et al., 1991). Petrasek et al. (2006) have recently shown that PIN auxin efflux proteins are sufficient to facilitate auxin efflux in yeast (*Saccharomyces cerevisiae*) cells, suggesting that directionality of auxin flow can be regulated by the subcellular localization of PIN proteins. The *PINOID* (*PID*) gene, encoding a protein Ser/Thr kinase, acts as a positive regulator of polar auxin transport (PAT) by regulating the subcellular localization of PIN1 (Bennett et al., 1995; Benjamins et al., 2001; Friml et al., 2004; Lee and Cho, 2006). Loss-of-function *pid* mutants display defects in lateral organ formation similar to *pin1* mutants, consistent with its role in regulating *PIN1*-mediated auxin efflux.

Auxin transport is promoted by the activity of the *MONOPTEROS* (*MP*) gene (Wenzel et al., 2007), which belongs to the auxin response factor (ARF) family of transcription factors (Guilfoyle et al., 1998; Hardtke and Berleth, 1998). Members of this family are post-translationally activated in response to auxin via auxin-mediated degradation of members of the AUX/IAA family of nuclear repressor proteins that bind to ARFs and inhibit ARF dimerization and subsequently target gene transcription (Kim et al., 1997; Ulmasov et al., 1997, 1999; Leyser and Berleth, 1999; Dharmasiri and Estelle, 2002; Liscum and Reed, 2002). Not only mutations in *PIN1* and *PID*, but also in the *MP* gene, interfere with lateral organ formation on inflorescence meristems (Przemeck et al., 1996). Local auxin application can restore flower formation on the flanks of *pin1* and *pid*, but not *mp* mutant inflorescences (Reinhardt et al., 2000, 2003), suggesting that in *mp* mutants, not the local supply of auxin, but auxin sensitivity, is diminished. Similarly, cotyledon response assays show that *mp* mutants are more resistant to the effects of exogenous auxin treatments than the strong auxin-resistant mutant allele *axr1-12*, demonstrating that *mp* mutants are severely defective in auxin signaling (Mattsson et al., 2003).

Recent reports show that ARFs, including *MP*, may regulate the expression of *PIN* genes (Sauer et al., 2006; Wenzel et al., 2007). To test whether *MP* exerts its effect on lateral organ formation exclusively as a regulator of *PIN* genes and auxin transport, we created *mp pin1* double mutants and also grew *mp* mutants on medium supplemented with auxin efflux inhibitors. Here, we show that *mp pin1* double mutants, as well as *mp* mutants treated with auxin efflux inhibitors, display strong synergistic abnormalities. These mutants fail to develop any lateral organs and the SAM develops into a leafless dome. The appearance of a synergistic defect indicates that the role of *MP* in shoot meristem organization is not limited to the regulation of auxin transport and the novel meristem phenotype implicates auxin transport and signaling in the regulation of meristem size.

RESULTS

mp pin1 Double Mutants Fail to Form Leaves

The shoot meristems of both *pin1* and *mp* single mutants produce a functional rosette of leaves from the vegetative SAM, but are highly defective in the analogous process of flower formation from the reproductive SAM (Okada et al., 1991; Przemeck et al., 1996; Fig. 1, A and B). To assess whether *MP* function in shoot organization acts exclusively through the regulation of auxin transport, we generated *mp pin1* double mutants. Analysis of progeny from a cross between heterozygous *mp* and *pin1* plants resulted in the identification of a fraction of *mp*-like plants that had formed a leafless dome from the SAM (Fig. 1, C and D). The segregation ratio of this novel phenotype was not significantly different from an expected theoretical value based on χ^2 analysis ($P = 0.75$; Supplemental Table S1), supporting the notion that the individuals were double mutants. The domes had a smooth surface and lacked differentiated epidermal, trichome, and stomata cells (Fig. 1D). After 2 to 3 weeks of culture in short-day conditions, the majority of the putative double mutants had developed additional leafless dome structures arising from the base of the initial dome (Fig. 1E). Such domes were never observed in single *mp* or *pin1* mutant populations. The appearance of a novel phenotype in the absence of both gene activities leads us to conclude that *MP* and *PIN1* act, at least in part, in separate pathways (see "Discussion").

Phenotypes of *mp pin1* double-mutant plants ranged from highly fasciated domes (Fig. 1F) to single or multiple dome formation and in the vast majority of all plants, leaf formation was absent. After 3 to 4 weeks of culture, many of the domes had formed one or more filament-like projections from its surface. A large number of these projections were formed after prolonged culture (Fig. 1, G and H). We interpreted these as inflorescences because they sometimes produced pistil-like or petal-like structures at their apices (Fig. 1I; data not shown). We found further evidence that *MP* acts on another pathway distinct from the regula-

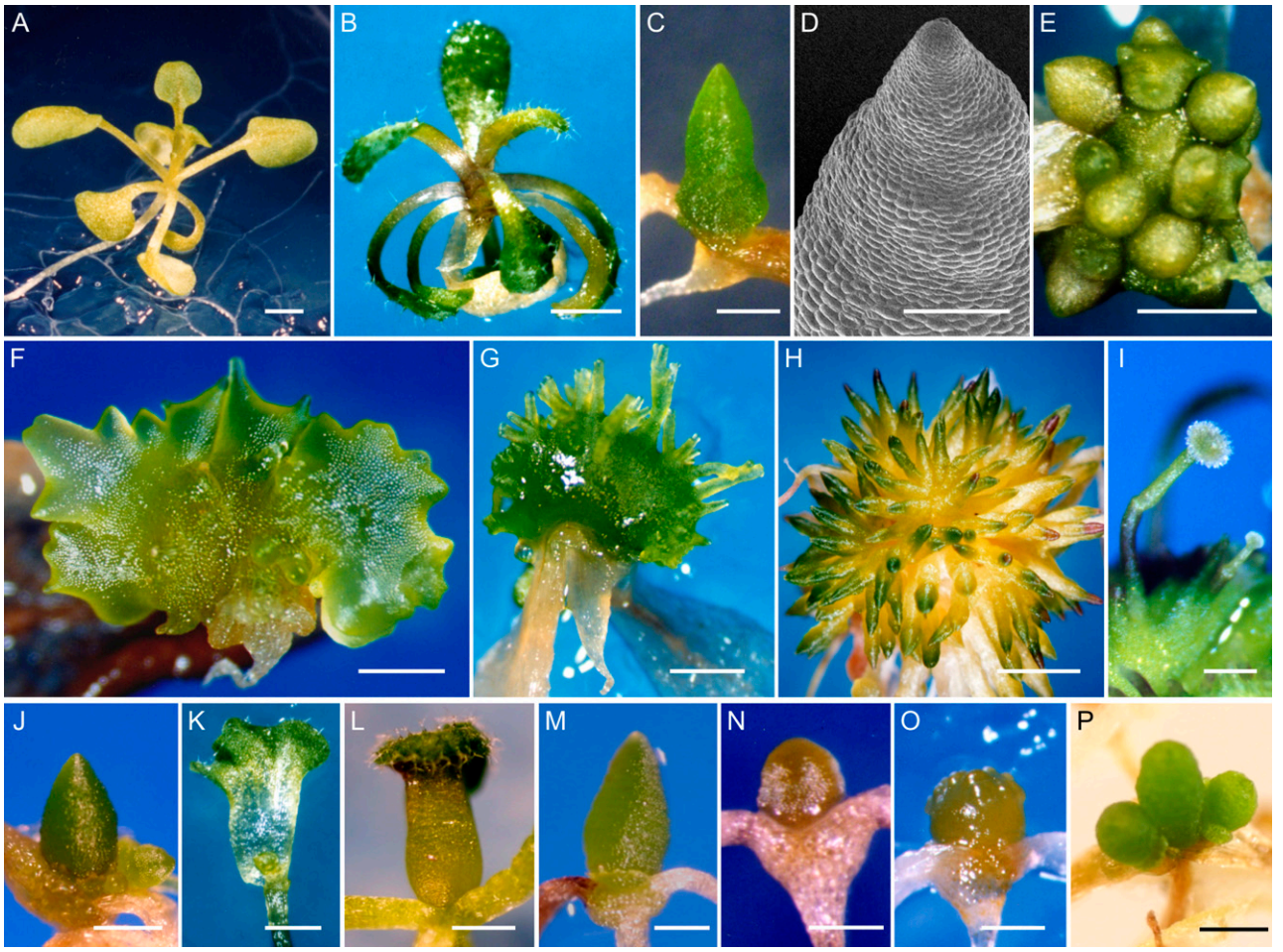

Figure 1. Development of leafless domes from *mp* meristems. A, Wild-type rosette of leaves at 14 DAG compared to *mp* at 21 DAG (B). C and D, Photograph (C) and scanning electron micrograph (D) of *mp pin1* double mutant at 40 DAG. E to I, *mp pin1* double mutants at 60 DAG (E and F) and 75 DAG (G and H). Multiple leafless domes (E) and example of extreme fasciation leading to leafless flattened structures (F). Examples of filamentous projections (G and H) sometimes ending in pistil-like structures (I). J, *mp pid* double mutant at 50 DAG. K, Single fused leaf and no cotyledons in a *pid pin1* double mutant at 14 DAG. L, A tubular third leaf in a 21-DAG wild-type seedling treated with 10 μM NPA. M to O, *mp* grown on medium with 10 μM NPA at 50 DAG (M), 40 μM 9-hydroxyfluorene-9-carboxylic acid at 35 DAG (N), and 40 μM 2,3,5-triiodobenzoic acid at 35 DAG (O). P, Leafless dome formation in Pro_{35S}:*MP* plant grown on medium with 10 μM NPA for 40 DAG. Scale bars = 1 mm (A and B); 500 μm (C, E–H, J–P); 100 μm (D); 50 μm (I).

tion of *PIN1* by the evaluation of *mp pid* and *pin1 pid* double mutants. The *PID* gene is known to be required for subcellular localization of PIN1 in plant cells transporting auxin (Friml et al., 2004) and may thus be thought to act in the same pathway as *PIN1*. Consistent with this interpretation, the *mp pid* double mutants produced phenotypes that were indistinguishable from the *mp pin1* phenotype (Fig. 1J; Supplemental Table S1). Further, as previously reported (Furutani et al., 2004), the *pin1 pid* double mutants were characterized by a variable degree of wide or fused leaves, but did not produce the leafless dome phenotype observed in *mp pin1* or *mp pid* double mutants. (Fig. 1K). The fact that the *pin pid* double mutant displays defects that are qualitatively similar to those of both single mutants is consistent with *PIN1* and *PID* acting in the same pathway, in line with molecular evidence (Friml et al., 2004). In summary, *mp pin1* and *mp pid* double mutants produced an identical, novel synergistic phenotype, suggesting that *MP* function in shoot meristem organization goes beyond the regulation of auxin transport processes (see "Discussion").

Reduction of Auxin Transport Does Not Abolish Lateral Organ Formation

The phenotypes from the *mp pin1* and *mp pid* double mutants suggest that, in the *mp* mutant background, leaf initiation becomes extremely sensitive to reduction of auxin transport. To assess this possibility, we grew *mp* seedlings on medium supplemented with the polar auxin efflux inhibitor 1-*N*-naphthylphthalamic acid (NPA). The observed defects very much resembled

the phenotype of *mp pin1* and *mp pid* double mutants (Fig. 1M). In addition, a large part of the heterogeneity observed in double mutants was lost at NPA concentrations at or above 10 μM NPA, suggesting that the heterogeneity was due to a comparatively weaker reduction in auxin transport in *pin1* or *pid* mutants. Similar phenotypes were obtained with other, chemically distinct, auxin efflux inhibitors (i.e. 9-hydroxyfluorene-9-carboxylic acid [HFCA] and 2,3,5-triiodobenzoic acid [TIBA]; Fig. 1, N and O) when applied to *mp* mutants.

Because auxin transport is reduced in *mp* mutants (Przemeck et al., 1996), we next asked whether the leafless dome phenotype could simply be a consequence of particularly weak auxin transport. To this end, we grew wild-type plants and *mp* mutants in the presence of increasing NPA concentrations. As shown in Figure 2A, leaf formation in wild type, but also in *pin1* and *pid* shoots, could not be abolished by any concentration of NPA, not even at 100 μM NPA, an eventually lethal concentration. Upon exposure to NPA, wild type, *pin1*, and *pid3* mutants developed leaf fusions or tubular leaves, but never formed leafless domes (Figs. 1L and 2A). In wild-type plants, 0.1 and 1 μM NPA had no significant effect on the numbers of leaves produced by 21 d after germination (DAG; Fig. 2B). In contrast, in *mp* mutants, NPA concentrations as low as 0.1 μM resulted in a dramatic decrease in leaf initiation (Fig. 2B) and, at concentrations of 1 μM NPA and higher, the majority of *mp* mutants developed leafless domes. The novel leafless domes continued to grow, demonstrating that their inability to produce leaves was not the expression of a general growth defect. We conclude that *MP*, in addition to promoting auxin transport, must stimulate another activity that leads to the actual formation and growth of leaf primordia (see "Discussion").

Plants with Ectopic Expression of MP Display Similar NPA Hypersensitivity

The above results suggest that a loss of *MP* function is required for the formation of the leafless dome phenotype in the presence of NPA. Because *MP* is expressed specifically in leaf anlagen and primordia at the flanks of the meristem (Hardtke and Berleth, 1998; Wenzel et al., 2007), we tested whether altered expression of MP would suffice to interfere with leaf formation under conditions of PAT inhibition. To this effect, we grew plants misexpressing *MP* from the constitutive cauliflower mosaic virus 35S promoter on medium supplemented with NPA. Growth of Pro_{35S}*:MP* plants in the presence of NPA did result in the frequent formation of leafless domes (Figs. 2A and 1P). The response of Pro_{35S}*:MP* plants to NPA was intermediate between wild type and *mp* mutants because leaf formation was abolished at 10 μM NPA in the majority of *mp* plants. Therefore, not only the expression of *MP* per se, but also its restriction to distinct domains, appears critical for the initiation of leaf primordia under conditions of reduced PAT.

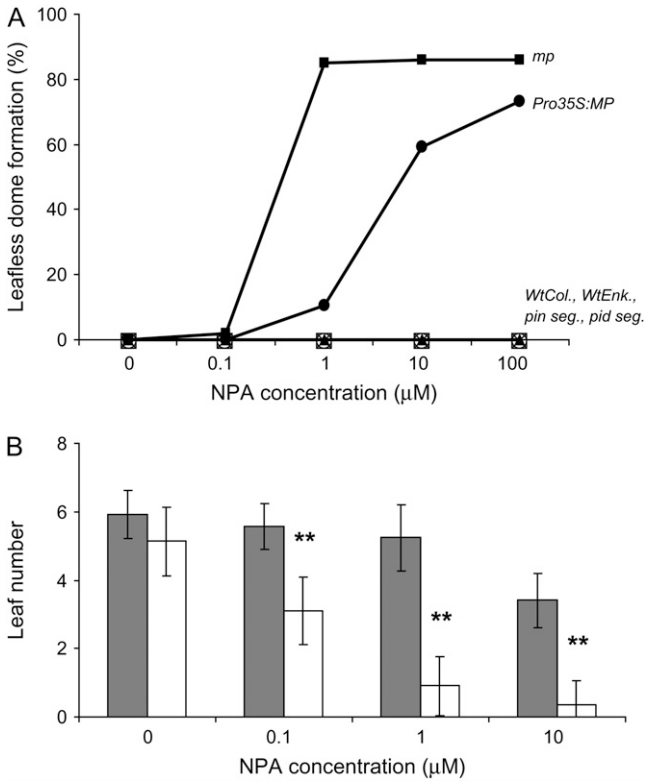

Figure 2. Frequency of leafless dome formation in response to NPA. A, Wild-type Columbia, wild-type Enkheim, *pin1*(*Enk*) segregating population, *pid* segregating population, Pro_{35S}*:MP*, and *mp* plants were grown on a series of medium containing 0 to 100 μM NPA. All genotypes were scored at 35 DAG and leafless dome formation was judged by the presence of leafless dome structure. Between 42 and 178 plants were scored for each genotype and treatment. B, Wild-type Columbia (gray bars) and *mp* (white bars) plants were grown on medium as in A and were scored for number of leaf primordia visible under a dissecting microscope at 21 DAG. **, Significant difference between NPA-grown wild type and *mp* mutants within the respective NPA treatment as determined by Student's *t* test analysis; $P < 0.05$. Error bars = SD.

SAM Enlarges during Leafless Dome Formation

The strict requirement of defects in *MP* activity for the formation of leafless domes led us to have a more careful look at the *mp* meristem and its ability to form leaves in the absence of PAT inhibition. We found various defects in phyllotaxy and growth of *mp* primordia compared to wild type (Fig. 3, A–C), suggesting that the *mp* meristem is already labile in this process. We also observed an immediate response of *mp* meristems to NPA. In the presence of 10 μM NPA, wild-type meristems initiated a normal first pair of leaf primordia, whereas *mp* meristems did not form any visible leaf primordia (Fig. 3, D and E). Instead, in *mp* mutants, the cells immediately surrounding the meristem appeared to elongate, forming a ring of elongated cells around the meristem (Fig. 3, E–G). Subsequently, the meristem region began to enlarge to initiate the formation of the leafless dome (Fig. 3, H and I).

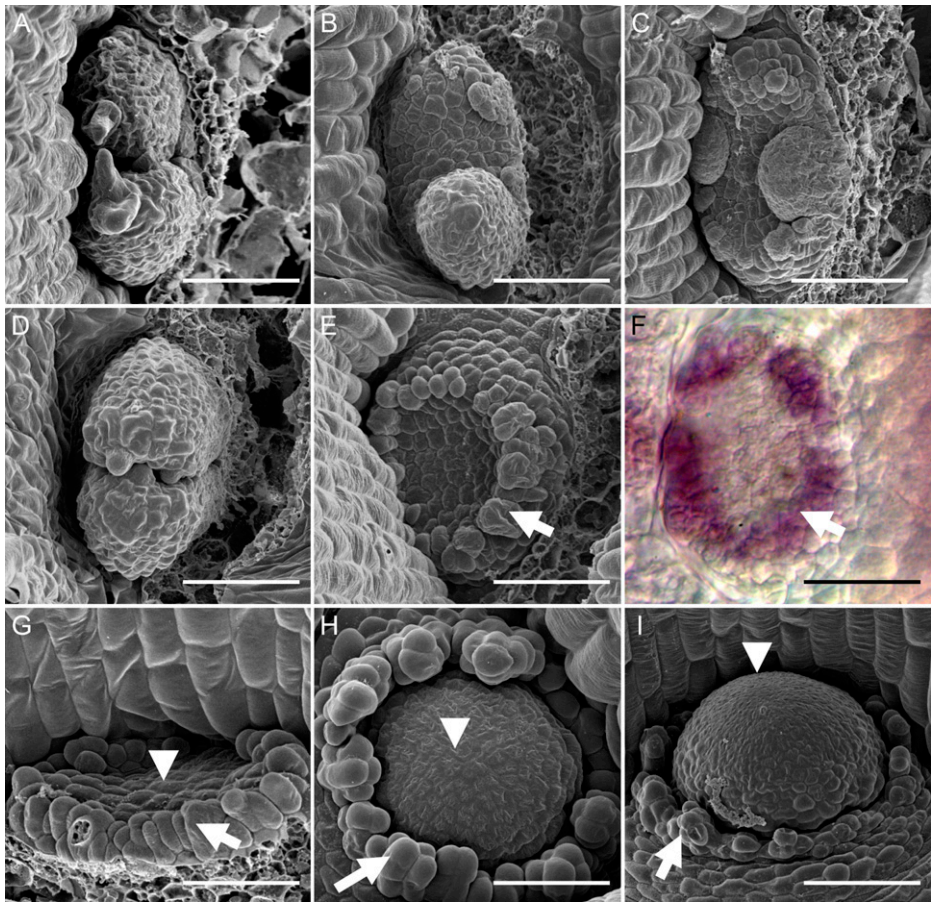

Figure 3. Phyllotactic defects in the *mp* meristem and the initiation of leafless domes. A to C, The first leaf primordia in wild-type seedlings (A) and *mp* mutants (B and C). *mp* mutants with two opposite cotyledons were used for analysis to preclude any effects of cotyledon placement on subsequent leaf primordia formation. D to I, Wild type (D) and *mp* mutants (E–I) grown on medium with 10 μM NPA. The cells in the peripheral region of the NPA-grown *mp* SAM elongate to form a collar of cells (E–I, arrows), which have leaf cell fate as judged by in situ hybridization with an *AS1* antisense probe (F). Central region of meristem indicated by arrowheads in G to I. All samples are 2 DAG, except H (4 DAG) and I (9 DAG). Scale bars = 50 μM. [See online article for color version of this figure.]

The ring of cells initially expressed the leaf founder cell marker *AS1* (Fig. 3F), but expression of *AS1* and growth of these cells ceased by 6 DAG (Supplemental Fig. S1). After approximately 4 to 6 DAG, all subsequent growth came from the meristem (Fig. 3, G–I) and the resulting leafless dome structure is derived entirely from this region.

To determine the extent and organization of the meristem domain in leafless domes, we assessed the expression conferred by the *STM* gene promoter in these structures. Figure 4, A to D, shows a comparison of Pro_{STM}:*GUS* meristem expression in wild-type and *mp* plants grown in the presence or absence of 10 μM NPA after 7 d of growth. Whereas the size of the meristem in 7-d-old wild-type, *mp* plants, and wild-type plants grown in the presence of NPA appeared comparable, the Pro_{STM}:*GUS* expression domain was more curved and visibly wider in NPA-grown *mp* plants (Fig. 4D). After 21 d of growth, a distinct leafless dome structure had developed in NPA-grown *mp* plants. The Pro_{STM}:*GUS* expression was localized at the apex of these structures (Fig. 4, F and G) and, although highly variable in size, appeared both wider and deeper than the corresponding expression domain in wild-type plants grown in parallel on the same medium (Fig. 4E). In NPA-grown *mp* plants, the leafless domes also expressed Pro_{STM}:*GUS* in thin strands along the apical-basal axis (Fig. 4F, arrows). Upon closer inspection, these strands appeared to consist of elongated and narrow cells typical of procambial strands (Fig. 4H). Similar procambial expression of *STM* has previously been reported in the pith meristem of wild-type plants (Long et al., 1996). The procambial strands, however, are frequently interrupted and never differentiate into vascular tissues (Fig. 4I; data not shown).

To further explore the enlarged SAMs in NPA-grown *mp* plants, we quantified the area of expression of the CZ marker, *CLV3*, and the meristem marker *STM* at 10 and 21 DAG using Pro_{CLV3}:*GFP* and Pro_{STM}:*GUS*, respectively (Fig. 5; representative imaged areas shown in Supplemental Fig. S2). After 10 d, the average areas of *CLV3* and *STM* expression were significantly larger in NPA-grown *mp* mutants. Although highly variable, the area of *CLV3* expression was on average 4.1 times and the *STM* expression 2.0 times as large in NPA-grown *mp* plants as compared to NPA-grown wild-type plants, thereby illustrating that leafless domes have enlarged CZs and meristem identity, respectively (Fig. 5, A and C). After 21 d of growth, the differences had increased further, with 5.5 times larger area of *CLV3* expression and 4.2 times larger area of *STM* expression in NPA-grown *mp* plants as compared to NPA-grown wild-type plants (Fig. 5, B and D). In summary, the leafless domes appear to have the organization of an enlarged shoot apex, comprising an

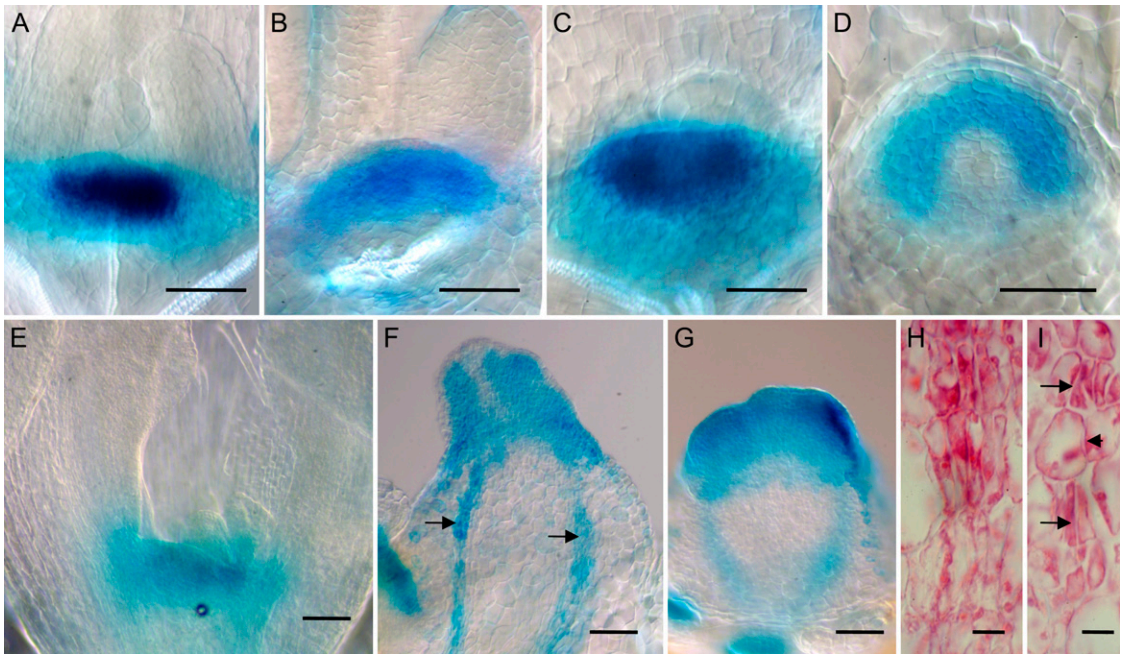

Figure 4. Expression of STM in leafless domes and procambial defects. Expression of Pro_{STM}:*GUS* in wild type (A), *mp* (B), wild type grown on medium with 10 μM NPA (C and E), and *mp* grown on medium with 10 μM NPA (D, F–I). Arrows in F indicate expression of Pro_{STM}:*GUS* in elongated procambial cell types orientated along the longitudinal axis. Longitudinal medial sections of leafless domes shows procambial strands (H and I) that are frequently interrupted (arrows in I). A to D, 7 DAG; E to I, 21 DAG. Size bars = 50 μm (A–G) and 10 μm (H and I).

apical meristem, and a basal radially organized stem region, but the CZ, as well as the entire meristem region, is enlarged and the basal region shows limited internal and external cellular differentiation.

Leafless Domes Fail to Focus *PIN1* Expression and Auxin

Previous studies have reported that *PIN1* expression is up-regulated at sites of flower primordia formation in the reproductive SAM (Heisler et al., 2005). We used a Pro_{PIN1}:*PIN1*:*GFP* marker to visualize *PIN1* expression in vegetative SAMs defective in *mp* and/or auxin transport functions. Our analysis showed that *PIN1* expression was most pronounced in discrete epidermal spots on the surface of vegetative wild-type SAMs and internal procambial midveins of young primordia (Fig. 6A), in agreement with previous findings from the reproductive SAM. In *mp* meristems, *PIN1* expression domains were more diffuse, occurred in defective phyllotactic patterns, and expression appeared spuriously in cells that are normally not involved in primordia formation (Fig. 6B). *PIN1* expression in NPA-grown wild-type seedlings was very weak or absent in the CZ area of the meristem, thereby forming a ring of high expression in the PZ, possibly predicting the future formation of a tubular leaf (Fig. 6C). Remarkably, in NPA-grown *mp* plants, *PIN1* expression was not even restricted to the PZ and, instead, expression was evenly distributed throughout the entire surface of young domes, including the CZ and more basal parts of the leafless dome (Fig. 6D). To assess whether the lack of *PIN1* focus formation in NPA-grown *mp* plants is accompanied by a lack of auxin maxima formation, we analyzed the expression of the auxin-responsive Pro_{DR5}:*GUS* marker. In wild-type seedlings, Pro_{DR5}:*GUS* is expressed initially at the apices of emerging leaf primordia and also internally in leaf primordia in conjunction with the formation of procambial tissues, but Pro_{DR5}:*GUS* expression is not found in the CZs and PZs of the SAM (Fig. 6E; Mattsson et al., 2003). In *mp* seedlings, the Pro_{DR5}:*GUS* expression in leaf primordia apices was always more diffuse than in wild-type seedlings (Fig. 6F). Wild-type plants responded to NPA with a considerable delay in leaf primordia formation and, when leaf primordia emerged, the Pro_{DR5}:*GUS* expression was found at the margins of the circular or close to circular leaf primordia (Fig. 6G). At no point did we observe localized Pro_{DR5}:*GUS* expression at the flanks of NPA-grown *mp* meristems (Fig. 6H). In summary, the leafless dome meristems of NPA-grown *mp* mutants show defects in the focusing of *PIN1* expression and do not form local auxin-response maxima as judged by Pro_{DR5}:*GUS*.

Leaf Founder Cell Markers Are Expressed in Leafless Dome Meristems

The synergistic phenotype in *mp pin1* double mutants suggests that *MP* acts not only through regulation of PAT in the process of leaf formation, but may separately promote the growth of leaf primordia.

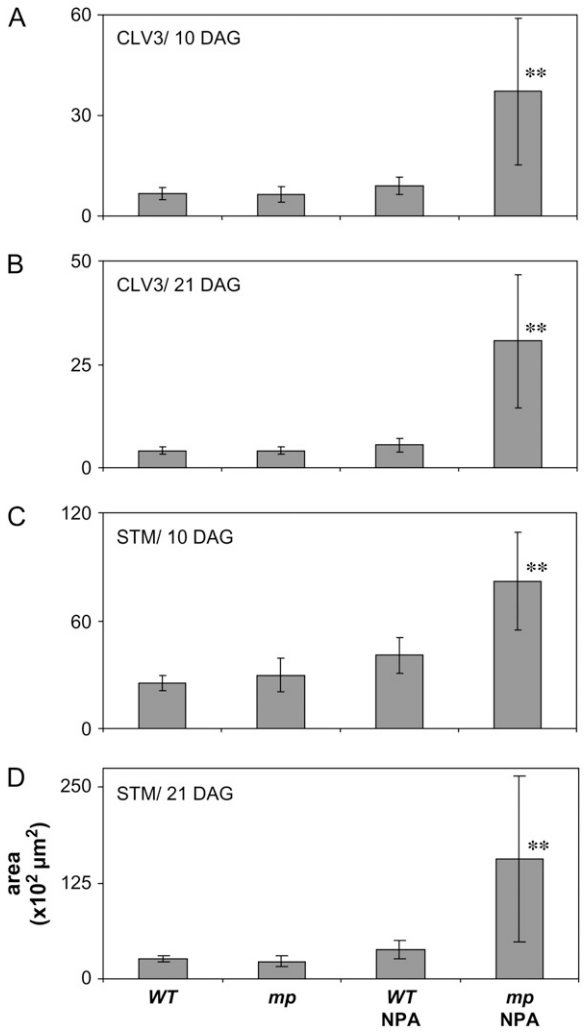

Figure 5. Quantification of CZ and meristem areas. Wild-type or *mp* plants were grown in the absence or presence of 10 μM NPA and the areas based on Pro_{CLV3}:*GFP:ER* expression domains at 10 (A) and 21 DAG (B), and Pro_{STM}:*GUS* expression domains at 10 (C) and 21 DAG (D). The *y* axis shows measured areas in 10^2 μm^2. Bars represent average of measured areas from six to 13 meristems; error bars are SD. **, Significant difference between NPA-grown *mp* mutants compared to all other genotypes and treatments, as determined by Student's *t* test analysis; $P < 0.05$. Representative images of measured areas are shown in Supplemental Figure S2.

Potential target genes could be involved in leaf founder cell fate specification or associated with subsequent organ outgrowth. The *ANT* and *AS1* genes are expressed in leaf founder cell populations and subsequently during outgrowth of leaf primordia (Elliott et al., 1996; Long and Barton, 1998; Byrne et al., 2000). We used the expression of these genes to assess whether leaf founder cell populations are established at the flanks of the meristem in leafless domes. In wild-type plants, we found that the expression of these markers preceded the formation of leaf primordia and that they were expressed in outgrowing primordia (Fig. 6, I and M), in agreement with published results. The expression of *ANT* and *AS1* in *mp* mutants appears identical to wild-type expression patterns (Fig. 6, J and N), except for the defects in phyllotaxy already described (Fig. 3, B and C). In response to NPA, wild-type plants expressed *ANT* and *AS1* in a circular domain (Fig. 6, K and O) consistent with the subsequent formation of a tubular leaf. We observed a similar ring-shaped expression of *ANT* and *AS1* near the apex of leafless domes in NPA-grown *mp* plants (Fig. 6, L and P). Thus, leaf founder cell populations appear to be specified in the PZs of wild-type and *mp* plants treated with NPA, but this specification is not sufficient for leaf formation in the latter. The failure to form leaves in leafless domes appears to be due to a defect in outgrowth of leaf primordia. In wild-type plants, early leaf initiation can be detected by a switch from anticlinal to periclinal cell divisions in the L2 layer (Medford et al., 1992). We screened longitudinal medial sections of more than 15 leafless domes without finding any indications of periclinal divisions in the L2 layer. Instead, we observed smooth surfaces of the PZ, and a pattern of cell walls in the L2 layer that indicated strict anticlinal cell division planes (Fig. 6, Q and R).

In summary, we conclude that the defect in leaf primordia formation in NPA-grown *mp* plants does not involve a block in the formation of leaf founder cells, but appears to involve a block of subsequent periclinal divisions in the process of leaf outgrowth, which appears to depend on *MP* activity.

DISCUSSION

Several lines of evidence have indicated that *PIN* gene expression is auxin (Heisler et al., 2005; Vieten et al., 2005, 2007; Scarpella et al., 2006; Wenzel et al., 2007) and ARF dependent (Sauer et al., 2006; Wenzel et al., 2007), suggesting that *MP* functions in leaf initiation by mediating *PIN* gene expression. In this case, however, one would expect that loss of *MP* function should not matter in plants severely compromised in auxin transport. Here, we observed that *mp* mutants of various allele strengths are hypersensitive to NPA treatment and display synergistic defects in double mutants with *pin1*. These findings provide strong evidence for an involvement of *MP* in a process beyond the control of auxin transport. Importantly, the synergistic defects cannot be mimicked by applying increased concentration of NPA to wild-type or *pin1* plants, further supporting that *MP* regulates further, hitherto unexplored, processes to promote leaf initiation. As one of those processes, we propose that *MP* has a role in promoting the actual outgrowth of leaves and flowers. Notably, it has also been suggested that, activating ARFs, including MP, could bind to the promoters of auxin-regulated leaf specification genes, thereby promoting leaf formation in the PZ of the meristem, whereas interaction with other ARFs limits this action in the CZ of the meristem (Leyser, 2006).

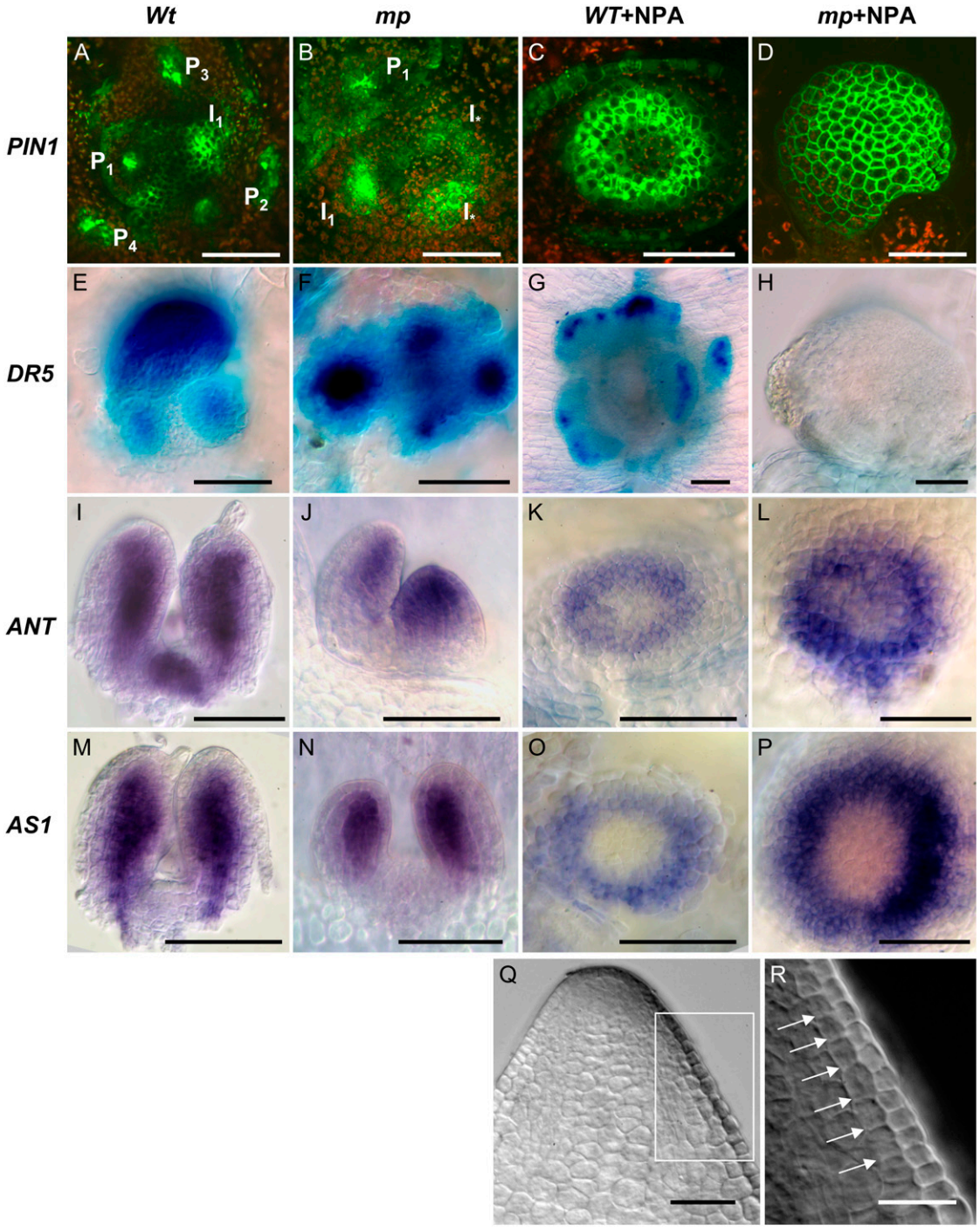

Figure 6. Marker analysis of leafless dome meristems. Material grown on medium supplemented with 10 μM NPA indicated as +NPA. A to D, Pro_{PIN1}:*PIN1*:*GFP* expression in wild type (A), *mp* (B), wild type + NPA (C), and *mp* NPA (D). Order of leaf primordia, present and incipient, is indicated in wild type (A) and *mp* (B). Aberrant expression is indicated by I*. E to H, Pro_{DR5}:*GUS* expression in wild type (E), *mp* (F), wild type + NPA (G), *mp* + NPA (H) at 21 DAG. I to L, *ANT* antisense probe at 3 DAG in wild type (I), *mp* (J), and 21-DAG wild type + NPA (K) and *mp* + NPA (L). M to P, *AS1* antisense probe at 3 DAG in wild type (M), *mp* (N), and 21-DAG wild type + NPA (O) and *mp* + NPA (P). Q and R, Apex of 21-DAG leafless dome (Q) and higher magnification of marked area in R. Arrows in R indicate cell walls produced by anticlinal cell divisions. All size bars = 50 μm, except for H (100 μm) and R (25 μm).

Given this scenario, ARFs like MP would therefore be implicated in also having functions in conferring differential properties to zones in the SAM.

Reinhardt et al. (2003) have formulated a model in which leaf primordia form at sites of elevated epidermal auxin concentration. Preexisting primordia are

thought to influence the position of new primordia by depleting the vicinity of auxin through auxin transport. Thus, new leaf primordia would only form at sites far enough away from existing primordia to allow new auxin maxima to form. This mechanism would not only explain the dependence of leaf formation on auxin maxima, but also the phyllotactic pattern of leaves and how it is influenced by the position of preexisting primordia. Mathematical modeling of leaf initiation based on these findings postulated a positive feedback loop concentrating auxin into concrete spots on the surface of the SAM because of positive influence of auxin on the amount and orientation of *PIN1* efflux carriers in neighboring cells (Jonsson et al., 2006; Smith et al., 2006). Mutants in the *PIN1* gene, as well as NPA-treated plants, fail to focus auxin through convergent *PIN1* polarity (Heisler et al., 2005) and do not form flowers from the inflorescence meristem (Okada et al., 1991; Vernoux et al., 2000). This is evidenced by the fact that, in NPA-treated plants, the concentration and polarization of PIN1-GFP toward individual spots is much reduced (Heisler et al., 2005). These interpretations are also consistent with the fact that flowers can be formed in both *pin1* mutant and NPA-treated inflorescence meristems upon local application of auxin. Apparently, the local application bypasses the need for auxin transport-driven focusing of auxin toward flower initiation sites.

MP is another likely component of the postulated mechanism because *mp* mutants also fail to form flowers from the inflorescence meristem and have reduced auxin transport capacity (Przemeck et al., 1996). Further, *MP* encodes an ARF (Ulmasov et al., 1997; Hardtke and Berleth, 1998), which might be involved in the auxin-dependent regulation of PIN expression (Sauer et al., 2006; Wenzel et al., 2007). No flowers can be induced by local auxin application on the flanks of *mp* inflorescence meristems (Reinhardt et al., 2003), suggesting that it is not only auxin transport and auxin accumulation that are defective in *mp* mutants, but also a failure to trigger lateral organ outgrowth even when auxin is locally provided (Reinhardt et al., 2003). Thus, published auxin application experiments already hint to a role of *MP* in controlling auxin responses in lateral organ outgrowth.

The inhibition of auxin transport in *mp* mutant backgrounds generates an unprecedented type of abnormal SAM development, which not only completely obstructs the formation of lateral organs, but also vastly expands the shoot apex. Marker gene expression indicates that the enlarged apical dome is composed of expanded *STM* and *CLV3*-expressing domains surrounded by a wide circular PZ, marked by *ANT* and *AS1*. Although no leaf primordia are formed under these conditions, there seems to be some dispersed growth because the *ANT* and *AS1* expression domains are extremely wide.

Under conditions of normal auxin transport, ARFs acting redundantly to *mp* appear to be sufficient for triggering organ formation from the vegetative, yet not from the reproductive, SAM as *mp* mutants produce leaves. Conversely, inhibition of auxin transport seems to allow for sufficient auxin focusing in the epidermis to trigger vegetative leaf initiation as long as *MP* is functional. However, poorly defined leaf initiation points seem to be insufficient to trigger organ outgrowth through redundantly acting ARFs when *MP* is not functional. Whereas failed leaf initiation may thus be explained as the superimposition of defects in two interdependent steps, the reasons for the enlargement of the CZ seem to reflect other, unknown levels of control. It has been proposed that the restriction of leaf-initiating auxin focusing to the PZ reflects auxin sensitivity zones due to the specific expression domains of competing ARFs (Leyser, 2006). In this interpretation, it is plausible that the removal of an important ARF may destabilize the zoning sufficiently to promote cell proliferation also in the CZ. In this context, it is remarkable that we observed equally strong PIN1-GFP expression in the PZ and CZ uniquely in NPA-exposed *mp* mutants. Formally, it is also possible that the expansion of the CZ could be a necessary consequence of defective lateral organ formation. Several levels of mutually antagonistic gene activities have been implicated in the control of stem cell pool size of the shoot meristem (for review, see Clark, 2001; Williams and Fletcher, 2005; Carraro et al., 2006; Tucker and Laux, 2007) in which some negative regulators originate from the PZ. Because there are no other leafless genotypes available, we cannot genetically separate leaflessness from SAM expansion. However, it should be noted that, in the inflorescences of *pin1* mutants devoid of lateral flowers, the size of the meristem and its constituent zones have been described as normal (Vernoux et al., 2000), arguing against a mechanism where signals negatively regulating shoot meristem size are derived from concrete flower or leaf primordia.

The sizes of SAMs vary considerably across the plant kingdom (Steeves and Sussex, 1989) and the influences of new regulators on SAM size are continuously being revealed (Chaudhury et al., 1993; Clark et al., 1993, 1995; Running et al., 2004; Green et al., 2005; Chiu et al., 2007). The discovery of highly abnormally sized SAMs as a consequence of simultaneous interference with auxin transport and ARF function may provide an entry point in the genetic analysis of auxin's role in this process.

MATERIALS AND METHODS

Plant Material and Growth

The $mp^{G12,\ G33,\ Tu399}$, *pid3*, and *pin1-1* mutant alleles used for double- and single-mutant analysis have been described previously by Okada et al. (1991), Berleth and Jurgens (1993), Hardtke and Berlet, (1998), Christensen et al. (2000), and Benjamins et al. (2001). All *MP* alleles used in this study are characterized as strong alleles and no differences were observed between different alleles and subsequent treatments or double-mutant generation. The *35S::MP* line was generated as described by Hardtke et al. (2004) and

overexpression of *MP* transcripts was confirmed by quantitative PCR using a Rotor-Gene 3000 real-time quantitative thermocycler (Corbett Life Sciences) and the Platinum SYBER Green quantitative PCR SuperMix (Invitrogen), with the primers MP-RT-F (CGATTTGGATCCGTTGAGAT) and MP-RT-R (ACCCCATTCAGTTTCACCAG; Hardtke et al., 2004; data not shown). The Pro_{DR5}::*GUS* (Ulmasov et al., 1997), Pro_{PIN1}:*PIN1*:*GFP* (Benkova et al., 2003), Pro_{STM}:*GUS* (Kirch et al., 2003), and Pro_{CLV3}:*GFP*:*ER* (Lenhard and Laux, 2003) transgenes were crossed into the *mp* mutant background. Attempts to introgress a Pro_{DR5}::*GFP* construct from the Arabidopsis Biological Resource Center into *mp* mutant background failed, possibly as a consequence of repulsion due to linkage. Surface-sterilized seeds were grown on ATS medium (Lincoln et al., 1990) and exposed to NPA as described (Mattsson et al., 1999). For quantification of *CLV3* and *STM* expression domains in meristems, images were taken and subsequently analyzed using ImageJ, version 1.37, software (National Institutes of Health). *mp* seedlings germinate approximately 1 d after wild type, most likely due to lack of hypocotyl and root. Comparable developmental stages were chosen for each dataset defined by the wild type; for example, the 3-DAG stage is defined as 3-DAG wild-type plants and 4-DAG *mp* plants, with similar sizes of leaf primordia.

In Situ Hybridization, Histology, and GUS Assays

All gene fragments were amplified from cDNA generated from total RNA extracted from 14-d-old wild-type seedlings using TRIzol reagent (Invitrogen) and subsequently reverse transcribed using RevertAid Moloney murine leukemia virus reverse transcriptase (Fermentas) and cloned into pBluescript II SK(−) (Stratagene). The *ANT* and *AS1* fragments were generated as described (Long and Barton, 1998; Byrne et al., 2000). Whole-mount in situ hybridization procedure was as described (Zachgo et al., 2000) with some modifications, including overnight fixation and agitation in a fresh solution containing 0.1 M triethanolamine (pH 8) and 0.5% (v/v) acetic anhydride for 15 min, followed by two washes in 1× phosphate-buffered saline solution prior to hybridization for 2 d at 60°C. For histological analysis, plant material was fixed and sectioned as described by Ruzin (1999). Localization of GUS activity was carried out as described by Mattsson et al. (2003).

Microscopy

A Zeiss LSM 410 was used to image Pro_{PIN1}:*PIN1*:*GFP* and Pro_{CLV3}:*GFP*:*ER* using a 488-nm excitation filter and 500- to 530-nm emission filter combination. Background red autofluorescence was detected using a 568-nm excitation filter and an LP 580 emission filter set. Digital interference contrast images were taken on a Nikon Eclipse 600 microscope using a Canon D30 digital camera and tissue clearing and preparation were performed as described by Mattsson et al. (1999). Samples for scanning electron micrographs were fixed in 2.5% glutaraldehyde in 0.05 M cacodylate buffer, dehydrated in a graded ethanol series before being critically point dried, and mounted on stubs. Samples were then coated with gold-palladium in a scanning electron microscope Prep2 sputter coater (Nanotech) and imaged using a Hitachi S-2600N VP-SEM.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Time series of *AS1* expression in leafless domes.

Supplemental Figure S2. Quantification of CZ and meristem areas.

Supplemental Table S1. Segregation of double mutants.

ACKNOWLEDGMENTS

We thank the Arabidopsis Biological Resource Center and Dr. Steven Chatfield (University of Guelph) for supplying marker lines, and the University of British Columbia bioimaging facility for excellent service. We also thank Dr. Sherryl Bisgrove and the reviewers of the manuscript for providing helpful suggestions to improve the text.

Received March 25, 2008; accepted July 2, 2008; published August 8, 2008.

LITERATURE CITED

Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M (1997) Genes involved in organ separation in *Arabidopsis*: an analysis of the cup-shaped cotyledon mutant. Plant Cell **9:** 841–857

Aida M, Ishida T, Tasaka M (1999) Shoot apical meristem and cotyledon formation during Arabidopsis embryogenesis: interaction among the CUP-SHAPED COTYLEDON and SHOOT MERISTEMLESS genes. Development **126:** 1563–1570

Benjamins R, Quint A, Weijers D, Hooykaas P, Offringa R (2001) The PINOID protein kinase regulates organ development in Arabidopsis by enhancing polar auxin transport. Development **128:** 4057–4067

Benkova E, Michniewicz M, Sauer M, Teichmann T, Seifertova D, Jurgens G, Friml J (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell **115:** 591–602

Bennett SRM, Alvarez J, Bossinger G, Smyth DR (1995) Morphogenesis in *pinoid* mutants of *Arabidopsis thaliana*. Plant J **8:** 505–520

Berleth T, Jurgens G (1993) The role of the Monopteros gene in organizing the basal body regions of the Arabidopsis embryo. Development **118:** 575–587

Byrne ME, Barley R, Curtis M, Arroyo JM, Dunham M, Hudson A, Martienssen RA (2000) Asymmetric leaves1 mediates leaf patterning and stem cell function in Arabidopsis. Nature **408:** 967–971

Byrne ME, Simorowski J, Martienssen RA (2002) ASYMMETRIC LEAVES1 reveals KNOX gene redundancy in Arabidopsis. Development **129:** 1957–1965

Carraro N, Peaucelle A, Laufs P, Traas J (2006) Cell differentiation and organ initiation at the shoot apical meristem. Plant Mol Biol **60:** 811–826

Chaudhury AM, Letham S, Dennis ES (1993) amp1: a mutant with high cytokinin levels and altered embryonic pattern, faster vegetative growth, constitutive photomorphogenesis, and precocious flowering. Plant J **4:** 907–916

Chiu WH, Chandler J, Cnops G, Van Lijsebettens M, Werr W (2007) Mutations in the TORNADO2 gene affect cellular decisions in the peripheral zone of the shoot apical meristem of Arabidopsis thaliana. Plant Mol Biol **63:** 731–744

Christensen SK, Dagenais N, Chory J, Weigel D (2000) Regulation of auxin response by the protein kinase PINOID. Cell **100:** 469–478

Chuck G, Lincoln C, Hake S (1996) KNAT1 induces lobed leaves with ectopic meristems when overexpressed in *Arabidopsis*. Plant Cell **8:** 1277–1289

Clark SE (2001) Cell signalling at the shoot meristem. Nat Rev Mol Cell Biol **2:** 276–284

Clark SE, Running MP, Meyerowitz EM (1993) CLAVATA1, a regulator of meristem and flower development in Arabidopsis. Development **119:** 397–418

Clark SE, Running MP, Meyerowitz EM (1995) CLAVATA3 is a specific regulator of shoot and floral meristem development affecting the same processes as CLAVATA1. Development **121:** 2057–2067

Dharmasiri S, Estelle M (2002) The role of regulated protein degradation in auxin response. Plant Mol Biol **49:** 401–409

Elliott RC, Betzner AS, Huttner E, Oakes MP, Tucker WQ, Gerentes D, Perez P, Smyth DR (1996) AINTEGUMENTA, an APETALA2-like gene of *Arabidopsis* with pleiotropic roles in ovule development and floral organ growth. Plant Cell **8:** 155–168

Fletcher JC, Brand U, Running MP, Simon R, Meyerowitz EM (1999) Signaling of cell fate decisions by CLAVATA3 in Arabidopsis shoot meristems. Science **283:** 1911–1914

Friml J, Yang X, Michniewicz M, Weijers D, Quint A, Tietz O, Benjamins R, Ouwerkerk PB, Ljung K, Sandberg G, et al (2004) A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. Science **306:** 862–865

Furutani M, Vernoux T, Traas J, Kato T, Tasaka M, Aida M (2004) PIN-FORMED1 and PINOID regulate boundary formation and cotyledon development in Arabidopsis embryogenesis. Development **131:** 5021–5030

Galweiler L, Guan C, Muller A, Wisman E, Mendgen K, Yephremov A, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. Science **282:** 2226–2230

Green KA, Prigge MJ, Katzman RB, Clark SE (2005) CORONA, a member of the class III homeodomain leucine zipper gene family in *Arabidopsis*, regulates stem cell specification and organogenesis. Plant Cell **17:** 691–704

Guilfoyle TJ, Ulmasov T, Hagen G (1998) The ARF family of transcription

factors and their role in plant hormone-responsive transcription. Cell Mol Life Sci **54:** 619–627

Hardtke CS, Berleth T (1998) The Arabidopsis gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. EMBO J **17:** 1405–1411

Hardtke CS, Ckurshumova W, Vidaurre DP, Singh SA, Stamatiou G, Tiwari SB, Hagen G, Guilfoyle TJ, Berleth T (2004) Overlapping and non-redundant functions of the Arabidopsis auxin response factors MONOPTEROS and NONPHOTOTROPIC HYPOCOTYL 4. Development **131:** 1089–1100

Heisler MG, Ohno C, Das P, Sieber P, Reddy GV, Long JA, Meyerowitz EM (2005) Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the Arabidopsis inflorescence meristem. Curr Biol **15:** 1899–1911

Hibara K, Takada S, Tasaka M (2003) CUC1 gene activates the expression of SAM-related genes to induce adventitious shoot formation. Plant J **36:** 687–696

Jonsson H, Heisler MG, Shapiro BE, Meyerowitz EM, Mjolsness E (2006) An auxin-driven polarized transport model for phyllotaxis. Proc Natl Acad Sci USA **103:** 1633–1638

Kim J, Harter K, Theologis A (1997) Protein-protein interactions among the Aux/IAA proteins. Proc Natl Acad Sci USA **94:** 11786–11791

Kirch T, Simon R, Grunewald M, Werr W (2003) The DORNROSCHEN/ENHANCER OF SHOOT REGENERATION1 gene of *Arabidopsis* acts in the control of meristem cell fate and lateral organ development. Plant Cell **15:** 694–705

Koyama T, Furutani M, Tasaka M, Ohme-Takagi M (2007) TCP transcription factors control the morphology of shoot lateral organs via negative regulation of the expression of boundary-specific genes in *Arabidopsis*. Plant Cell **19:** 473–484

Kumaran MK, Bowman JL, Sundaresan V (2002) YABBY polarity genes mediate the repression of KNOX homeobox genes in *Arabidopsis*. Plant Cell **14:** 2761–2770

Lee SH, Cho HT (2006) PINOID positively regulates auxin efflux in *Arabidopsis* root hair cells and tobacco cells. Plant Cell **18:** 1604–1616

Lenhard M, Laux T (2003) Stem cell homeostasis in the Arabidopsis shoot meristem is regulated by intercellular movement of CLAVATA3 and its sequestration by CLAVATA1. Development **130:** 3163–3173

Leyser O (2006) Dynamic integration of auxin transport and signalling. Curr Biol **16:** R424–433

Leyser O, Berleth T (1999) A molecular basis for auxin action. Semin Cell Dev Biol **10:** 131–137

Lincoln C, Britton JH, Estelle M (1990) Growth and development of the axr1 mutants of *Arabidopsis*. Plant Cell **2:** 1071–1080

Liscum E, Reed JW (2002) Genetics of Aux/IAA and ARF action in plant growth and development. Plant Mol Biol **49:** 387–400

Long J, Barton MK (2000) Initiation of axillary and floral meristems in Arabidopsis. Dev Biol **218:** 341–353

Long JA, Barton MK (1998) The development of apical embryonic pattern in Arabidopsis. Development **125:** 3027–3035

Long JA, Moan EI, Medford JI, Barton MK (1996) A member of the KNOTTED class of homeodomain proteins encoded by the STM gene of Arabidopsis. Nature **379:** 66–69

Mattsson J, Ckurshumova W, Berleth T (2003) Auxin signaling in Arabidopsis leaf vascular development. Plant Physiol **131:** 1327–1339

Mattsson J, Sung ZR, Berleth T (1999) Responses of plant vascular systems to auxin transport inhibition. Development **126:** 2979–2991

Medford JI, Behringer FJ, Callos JD, Feldmann KA (1992) Normal and abnormal development in the *Arabidopsis* vegetative shoot apex. Plant Cell **4:** 631–643

Meicenheimer RD (1981) Changes in *Epilobium* phyllotaxy induced by *N*-1-naphthylphthalamic acid and a-4-chlorophenoxyisobutyric acid. Am J Bot **68:** 1139–1154

Mizukami Y, Fischer RL (2000) Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. Proc Natl Acad Sci USA **97:** 942–947

Muday GK, DeLong A (2001) Polar auxin transport: controlling where and how much. Trends Plant Sci **6:** 535–542

Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y (1991) Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. Plant Cell **3:** 677–684

Ori N, Eshed Y, Chuck G, Bowman JL, Hake S (2000) Mechanisms that control KNOX gene expression in the Arabidopsis shoot. Development **127:** 5523–5532

Petrasek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, Seifertova D, Wisniewska J, Tadele Z, Kubes M, Covanova M, et al (2006) PIN proteins perform a rate-limiting function in cellular auxin efflux. Science **312:** 914–918

Przemeck GK, Mattsson J, Hardtke CS, Sung ZR, Berleth T (1996) Studies on the role of the Arabidopsis gene MONOPTEROS in vascular development and plant cell axialization. Planta **200:** 229–237

Reddy GV, Heisler MG, Ehrhardt DW, Meyerowitz EM (2004) Real-time lineage analysis reveals oriented cell divisions associated with morphogenesis at the shoot apex of Arabidopsis thaliana. Development **131:** 4225–4237

Reinhardt D, Mandel T, Kuhlemeier C (2000) Auxin regulates the initiation and radial position of plant lateral organs. Plant Cell **12:** 507–518

Reinhardt D, Pesce ER, Stieger P, Mandel T, Baltensperger K, Bennett M, Traas J, Friml J, Kuhlemeier C (2003) Regulation of phyllotaxis by polar auxin transport. Nature **426:** 255–260

Running MP, Lavy M, Sternberg H, Galichet A, Gruissem W, Hake S, Ori N, Yalovsky S (2004) Enlarged meristems and delayed growth in plp mutants result from lack of CaaX prenyltransferases. Proc Natl Acad Sci USA **101:** 7815–7820

Ruzin SE (1999) Plant Microtechnique and Microscopy. Oxford University Press, New York

Sauer M, Balla J, Luschnig C, Wisniewska J, Reinohl V, Friml J, Benkova E (2006) Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. Genes Dev **20:** 2902–2911

Scarpella E, Marcos D, Friml J, Berleth T (2006) Control of leaf vascular patterning by polar auxin transport. Genes Dev **20:** 1015–1027

Schoof H, Lenhard M, Haecker A, Mayer KF, Jurgens G, Laux T (2000) The stem cell population of Arabidopsis shoot meristems in maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. Cell **100:** 635–644

Shani E, Yanai O, Ori N (2006) The role of hormones in shoot apical meristem function. Curr Opin Plant Biol **9:** 484–489

Smith RS, Guyomarc'h S, Mandel T, Reinhardt D, Kuhlemeier C, Prusinkiewicz P (2006) A plausible model of phyllotaxis. Proc Natl Acad Sci USA **103:** 1301–1306

Steeves TA, Sussex IM (1989) Patterns in Plant Development. Cambridge University Press, Cambridge, UK

Tucker MR, Laux T (2007) Connecting the paths in plant stem cell regulation. Trends Cell Biol **17:** 403–410

Ulmasov T, Hagen G, Guilfoyle TJ (1999) Dimerization and DNA binding of auxin response factors. Plant J **19:** 309–319

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell **9:** 1963–1971

Vernoux T, Kronenberger J, Grandjean O, Laufs P, Traas J (2000) PIN-FORMED 1 regulates cell fate at the periphery of the shoot apical meristem. Development **127:** 5157–5165

Vieten A, Sauer M, Brewer PB, Friml J (2007) Molecular and cellular aspects of auxin-transport-mediated development. Trends Plant Sci **12:** 160–168

Vieten A, Vanneste S, Wisniewska J, Benkova E, Benjamins R, Beeckman T, Luschnig C, Friml J (2005) Functional redundancy of PIN proteins is accompanied by auxin-dependent cross-regulation of PIN expression. Development **132:** 4521–4531

Vroemen CW, Mordhorst AP, Albrecht C, Kwaaitaal MA, de Vries SC (2003) The CUP-SHAPED COTYLEDON3 gene is required for boundary and shoot meristem formation in *Arabidopsis*. Plant Cell **15:** 1563–1577

Wardlaw CW (1949) Experiments on organogenesis in ferns. Growth **13:** 93–131

Wenzel CL, Schuetz M, Yu Q, Mattsson J (2007) Dynamics of MONOPTEROS and PIN-FORMED1 expression during leaf vein pattern formation in Arabidopsis thaliana. Plant J **49:** 387–398

Williams L, Fletcher JC (2005) Stem cell regulation in the Arabidopsis shoot apical meristem. Curr Opin Plant Biol **8:** 582–586

Zachgo S, Perbal MC, Saedler H, Schwarz-Sommer Z (2000) In situ analysis of RNA and protein expression in whole mounts facilitates detection of floral gene expression dynamics. Plant J **23:** 697–702