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AMP1 and *MP* antagonistically regulate embryo and meristem development in *Arabidopsis*

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AUXIN RESPONSE FACTOR (ARF)-mediated signaling conveys positional information during embryonic and postembryonic organogenesis and mutations in *MONOPTEROS* (*MP*/*ARF5*) result in severe patterning defects during embryonic and postembryonic development. Here we show that *MP* patterning activity is largely dispensable when the presumptive carboxypeptidase *ALTERED MERISTEM PROGRAM 1* (*AMP1*) is not functional, indicating that *MP* is primarily necessary to counteract *AMP1* activity. Closer inspection of the single and double mutant phenotypes reveals antagonistic influences of both genes on meristematic activities throughout the *Arabidopsis* life cycle. In the absence of *MP* activity, cells in apical meristems and along the paths of procambium formation acquire differentiated identities and this is largely dependent on differentiation-promoting *AMP1* activity. Positions of antagonistic interaction between *MP* and *AMP1* coincide with MP expression domains within the larger AMP1 expression domain. These observations suggest a model in which auxin-derived positional information through *MP* carves out meristematic niches by locally overcoming a general differentiation-promoting activity involving *AMP1*.

KEY WORDS: *amp1*, *Arabidopsis*, Embryogenesis, Meristem, *mp*, Stem cells

INTRODUCTION

Cell division is unequally distributed in the plant body. Growth regions with controlled patterns of dividing cells, termed apical meristems, elongate the plant axis and an extended meristem, the procambium, retains pluripotent cells for subsequent vascular differentiation. In many locations, proliferative activity is inversely correlated with the differentiation status of cells and their balance defines the sizes of meristematic regions. In some meristematic tissues, as for example in the procambium, dividing cells give rise to limited cell numbers in specialized tissues (Esau, 1965), whereas in apical meristems permanent stem cells give off daughter cells indefinitely (Weigel and Jürgens, 2002).

The controls regulating the balance between proliferating and differentiating cells are only partially understood. Where amenable to genetic dissection, as in the shoot apical meristem (SAM), these controls seem to comprise antagonistic activities acting in specific zones (reviewed by Bäurle and Laux, 2003; Williams and Fletcher, 2005). Antagonistic activities might also control the size of other meristems. A mechanism related to that in the SAM has been proposed for the root meristem (Casamitjana-Martinez et al., 2003), and the formation of procambium in the leaf seems to occur in competition with mesophyll differentiation (Scarpella et al., 2004).

Mutations in the presumptive glutamate carboxypeptidase *AMP1* are associated with diverse morphological abnormalities including supernumerary cotyledons, shortened plastochrons and a bushy appearance, and are further characterized by cytokinin overproduction and upregulation of *CYCD3;1* (Chaudhury et al., 1993; Chin-Atkins et al., 1996; Nogué et al., 2000a; Nogué et al., 2000b; Riou-Khamlichi et al., 1999). However, *amp1* mutants are phenotypically distinct from both cytokinin or CYCD3;1-overproducing plants and it is unclear what primary defect could account for the various aspects of the *amp1* phenotype. Despite a wealth of phenotypic data, *AMP1* function has not been genetically linked to other genes in embryo or meristem patterning. The AMP1 product bears similarities to mammalian *N*-acetyl α-linked acidic dipeptidases (NAALADases) (Helliwell et al., 2001), but neither its organismal or cellular localization nor the molecular identity of its targets is known.

Auxin distribution patterns have been implicated in positioning of lateral organs in shoots and roots (Reinhardt et al., 2003; Benkova et al., 2003), the formation of vascular tissues (Aloni et al., 2003; Avsian-Kretchmer et al., 2002; Mattsson et al., 2003) and in the generation of the root stem cell niche (Aida et al., 2004). In all these positions, robust patterns of auxin accumulation were found associated with patterned cell fate specification, including positioning of meristematic activities. Other plant hormones, specifically cytokinins, are also essential for promoting cell division (Bishopp et al., 2006), but their distribution patterns have not been as precisely correlated to cellular responses. Auxin regulates gene expression through auxin response factors (ARFs) and their nuclear co-regulators of the Aux/IAA family (Guilfoyle and Hagen, 2001; Liscum and Reed, 2002). Although most ARF functions are still elusive, patterning functions involving organ initiation and growth have been assigned to some ARFs, including *MONOPTEROS* (*MP*/*ARF5*). Mutations in *MP* lead to the absence of an embryonic root, the formation of reduced vascular systems and flowerless shoots (Berleth and Jürgens, 1993; Przemeck et al., 1996). Among other ARFs, *ARF7*/*NON-PHOTOTROPIC HYPOCOTYL 4* and *ARF19* are required for local cell proliferation in the pericycle to produce lateral roots (Okushima et al., 2005a; Wilmoth et al., 2005). By contrast, *ARF2* has been shown to restrict the size of *Arabidopsis* ovules and seeds and to negatively regulate certain cell proliferation genes (Ellis et al., 2005; Okushima et al., 2005b; Schruff et al., 2005).

Here we identify *amp1* as a first loss-of-function suppressor of an *arf* mutant and present evidence that *AMP1* has a role in balancing and restricting the meristem-promoting activity of auxin signaling. We document that *MP* has an important role in promoting meristematic niches in diverse locations and that this activity is dispensable in the absence of a counteracting pathway involving *AMP1*.

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MATERIALS AND METHODS

Plant material and growth conditions

Unless otherwise noted, seeds were plated and plants grown as previously described (Hardtke et al., 2004). Origin of transgenic lines: *CycB1;1::CycB1;1-GUS* (Donnelly et al., 1999), *pCLV3::GUS* (Brand et al., 2002), *SNO-GFP* (Cutler et al., 2000). *UBI3::Lti6b-GFP* was generated by M. Aida in the laboratory of B. Scheres (Utrecht University, Utrecht, The Netherlands) by fusion of membrane marker 29-1 (Cutler et al., 2000) to the potato *UBI3* promoter (L22576).

Microtechniques and microscopy

Cleared whole-mount samples were prepared as described in Berleth and Jürgens (Berleth and Jürgens, 1993). Detection of β-glucuronidase (GUS) activity was as in Scarpella et al. (Scarpella et al., 2004) with the following modifications to the concentration of potassium ferro- and ferricyanide and incubation times: 10 mM for 1 hour (*pCLV3::GUS*), 0.5 mM for 16 hours (*MP::MP-GUS* embryos), 2 mM for 2 hours (*CycB1;1:CycB1;1-GUS*), 5 mM for 2 hours (*MP::MP-GUS* seedlings) or 5 mM plus 1% Triton X-100 for 1 hour (*MP::MP-GUS* nuclear localization). Scanning electron microscopy and confocal laser scanning microscopy were performed as described (Douglas et al., 2002; Gazzarrini et al., 2004).

Sizes of inflorescence meristems were determined on images taken from above the meristem by measuring the distance from the centre of the youngest recognizable floral primordium to the centre of the furrow separating the fifth flower primordium from the meristem as described (Yu et al., 2000). SAM sizes were determined on cleared medium longitudinal images using ImageJ 1.33 software (http://rsb.info.nih.gov/ij/) as being the area formed by the dome of the meristem connected by a straight line between the cotyledon primordia.

RESULTS AND DISCUSSION

A survey of the molecular lesions and phenotypic strengths of *amp1* mutations, including six new alleles, identified *amp1-10* and *amp1-13* as likely null alleles with no recognizable *AMP1* transcripts and *amp1-1* as an allele with pronounced residual gene activity (see Fig. S1 and Table S1 in the supplementary material). As there are no apparent *AMP1* paralogs in the *Arabidopsis* genome, the two allele-strength categories probably reflect partial and complete loss of NAALADase activity in the *AMP1* pathway.

AMP1 function stabilizes suspensor cell fate and restricts cell numbers in embryos

Cell numbers and cell division patterns in the early wild-type *Arabidopsis* embryo are almost invariable. A particularly reproducible feature of the *Arabidopsis* embryonic fate map is the restriction of the descendents of the apical and basal daughter cells of the zygote. Basal cell descendents form the suspensor, but, except for the central portion of the root meristem, they do not contribute to the seedling pattern (Scheres et al., 1994). In *amp1* embryos, abnormal divisions of basal cell derivatives gave rise to additional cell tiers in the embryo proper and basal cell derivatives regularly contributed to large parts of the seedling, including the hypocotyl and cotyledons (Fig. 1I,J,P-S; frequency of extra tiers in *amp1-13*: 36/42, 42/54, 63/63, 96/96 at 4-, 8-, 16-cell and globular stages, respectively). Conversely, cells from the apical part of the globular embryo (framed cells in Fig. 1G,H,I) no longer contributed to cotyledons but became incorporated into an oversized SAM (Fig. 1Q,R,S). At lower frequency, abnormal divisions of basal derivatives led to the formation of a complete second embryo from the same zygote, which was reflected in the appearance of twin seedlings from single seeds in *amp1* mutant lines (Fig. 1V,W and see Table S1B in the supplementary material). Except for the oversized shoot meristem and frequent supernumerary cotyledons, the architecture of *amp1* late-stage embryos is remarkably normal (Fig. 1Y) (Conway and Poethig, 1997). These features suggest that the mutant phenotype is primarily a consequence of the increased cell numbers in early pro-embryos.

In conclusion, the patterning defects in *amp1* mutant embryos can be traced back to the failure of basal cell descendents to attain suspensor cell fate. Instead of displaying suspensor-specific differentiation features, some of those cells proliferate and either generate additional embryos or contribute to inappropriately large portions of the embryo proper.

AMP1 negatively regulates meristematic activities in shoots and roots

The enlarged SAM is not solely a consequence of abnormal cell specification in the embryo. As shown in Fig. 2B,F,J and Table 1A, in *amp1* mutants, SAMs continued to increase in diameter post-embryonically and were enlarged in the central stem cell regions, as visualized by expression of *pCLV3::GUS*. In addition, expression of *pCLV3::GUS* was generally extended towards the flanks of the SAM (Fig. 2F). In extreme cases, the *pCLV3::GUS* expression domain was five times wider than in wild type and became concentrated in concrete spots (Fig. 2M). These spots might be correlated with the formation of multiple SAMs (Fig. 2N), which we observed in all *amp1* alleles, reminiscent of what has been described for the *corona* mutant (Green et al., 2005). By contrast, the sizes of the *amp1* inflorescence and floral meristems were not markedly abnormal [mean inflorescence meristem diameter±s.e.m.: wild type, 52.6±0.7 mm (n=16); *amp1-9*, 51.4±1.2 mm (n=12)] (Fig. 2V,W).

In hypocotyls and roots, pericycle cells retain proliferation competence, but only a portion of them will later progress through the cell cycle to produce lateral roots (Beeckman et al., 2001; Himanen et al., 2002). In *amp1* mutants, the proportion of pericycle cells that were actually proliferating was greatly increased. Lateral roots were initiated very early (Fig. 2AA, Table 1C) and adventitious roots from hypocotyls were frequent (Fig. 2AE, Table 1B). Once established, *amp1* mutant root meristems were not expanded in diameter (data not shown).

In summary, our results document that *AMP1* restricts stem cell pool sizes in the SAM and keeps division-competent cells dormant in the pericycle.

MP promotes meristem formation in roots and shoots

Mutations in *mp* are associated with the absence of an embryonic root (Berleth and Jürgens, 1993) and *MP* has been implicated in the generation of a stem cell niche in the root meristem (Aida et al., 2004). As shown in Fig. 2D,H,L, *MP* was found to promote stem cell formation not only in the root meristem, but also in the SAM. In wild type, the SAM is initiated during embryogenesis and produces the first two leaf primordia approximately at the time of germination (Laux et al., 1996). In *mp* mutants, the SAM was typically not visible at germination and SAMs were also smaller in *mp* seedlings (Fig. 2D,H,L, Table 1A). As previously reported, mutant inflorescence meristems are unable to produce normal numbers of flowers, floral meristems produce fewer floral organs (Fig. 2U,Y) (Przemeck et al., 1996) and *mp* mutants have incomplete vascular systems (Fig. 3A). This defect has been traced back to a reduced procambium, the meristematic precursor tissue of vascular strands (Przemeck et al., 1996). Finally, we found that *mp* mutants produce adventitious roots only after many weeks in culture, in sharp contrast to the enhanced production of adventitious roots in *amp1* mutants (Table 1B). In summary, *mp* mutants are defective in the generation of appropriately sized meristems in various locations.

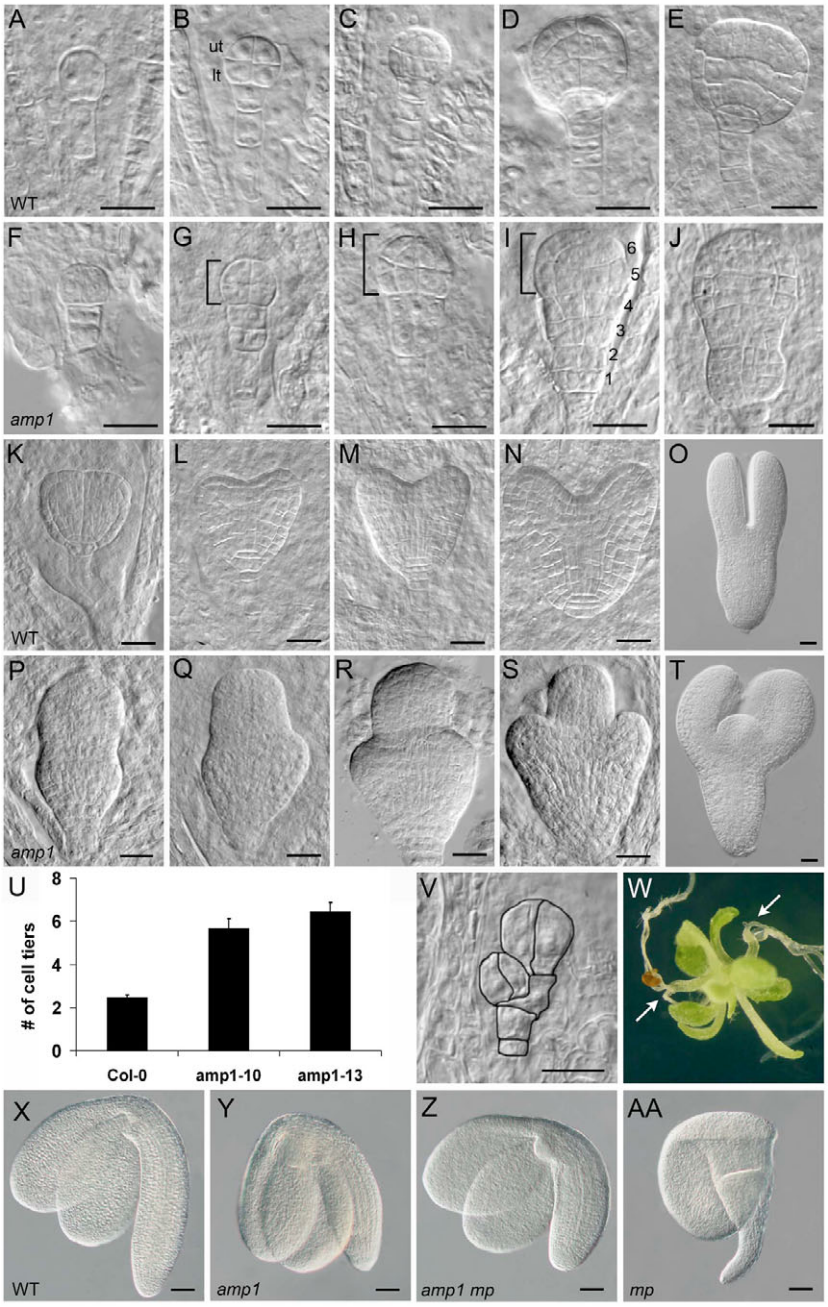

Fig. 1. *Arabidopsis* embryo development. Quadrant (**A**,**F**), octant (**B**,**G**), dermatogen (**C**,**H**) globular (**D**,**E**,**I**,**J**), and postglobular (**K-T**) stages of phenotypically normal (A-E,K-O) and *amp1-13* (F-J,P-T) embryos. Staging of *amp1* mutants was based on comparison with phenotypically normal (*amp1*/+ or +/+) embryos from the same silique. Abnormalities in preglobular *amp1* mutant embryos are restricted to basal-cell derivatives, whereas the arrangement of apical derivatives (brackets in G-I) remains unaltered. Abnormal suspensor cell divisions (F) lead to a massive three-dimensional cell arrangement (I,J), clearly recognizable as part of the embryo proper by the triangular stage (L,Q). Note the presence of a basally extended epidermal layer (I) and the presence of additional cell tiers in the embryo proper in *amp1* mutants (numbered in I). Note the emergence of cotyledon primordia from basal positions and of an oversized SAM at triangular (P), heart (Q-S) and torpedo (T) stage in *amp1* mutants. (**U**) Quantification of cell tier numbers (as illustrated in I) for wild-type and *amp1* globular-stage embryos (*n* values between 42 and 51; error bars indicate s.e.m.). (**V**,**W**) Formation of a second embryo (outlined cells) and of twin-seedlings from single seeds in *amp1-13* mutant seeds. Arrows in W point to two separate roots. (**X-AA**) Bent-cotyledon-stage embryos of wild-type (X), *amp1-9* (Y), *amp1-9 mpG92* (Z) and *mpG92* (AA) genotype. The embryo in Z represents a largely normalized individual from a spectrum of embryonic phenotypes. All images except W are cleared whole-mounts viewed with DIC optics. ut, upper tier; lt, lower tier. Scale bars: 20 μm in A-J,V; 50 μm in K-T,X-AA.

amp1 uncouples embryo and meristem development from *MP* dependence

We assessed the possibility that *MP* and *AMP1* activity antagonize each other in the control of meristematic activities by constructing *amp1 mp* double mutants of various allelic combinations. We found that *amp1* suppresses the phenotype of *mp* and can even restore viability and fertility in an *mp* mutant background. Whereas rootless *mp* mutant seedlings were not viable under normal growth conditions, *amp1 mp* double mutants frequently formed hypocotyls and roots (Fig. 1Z, Fig. 2AB, Table 1D) and could be grown on soil (Fig. 2O-Q). In fact, *amp1 mp* double-mutant embryo development could be indistinguishable from *amp1* embryogenesis (data not shown). Further, in contrast to the generally flower-defective and invariably sterile *mp* mutants, inflorescences of *amp1 mp* double mutants had abundant fertile flowers with partially restored numbers of floral organs (Fig. 2T,X, Table 1E). Mutations in *AMP1* also increased the reduced cotyledon numbers in *mp* mutants (Table 1F) and we observed a restoration of adventitious root formation in *amp1 mp* double mutants (Table 1B). Finally, loss of *AMP1* function significantly restored vascular tissue formation in the *mp* mutant background (Fig. 3A). Whereas the mature vascular system in cotyledons of *mp* mutants was typically restricted to a short midvein and occasional short side branches, cotyledon venation of *amp1 mp* double mutants comprised several lateral circular veins similar to wild-type cotyledons.

Suppression of *mp* by *amp1* mutations was observed in a variety of allelic combinations. The resulting phenotypes support the notion of quantitative antagonistic activities, because the suppression of *mp* was generally weaker in combinations involving the weak *amp1-1* allele (Fig. 2P,Q, Table 1D). In summary, our results indicate that *MP* and *AMP1* genetically interact in the regulation of meristematic activity.

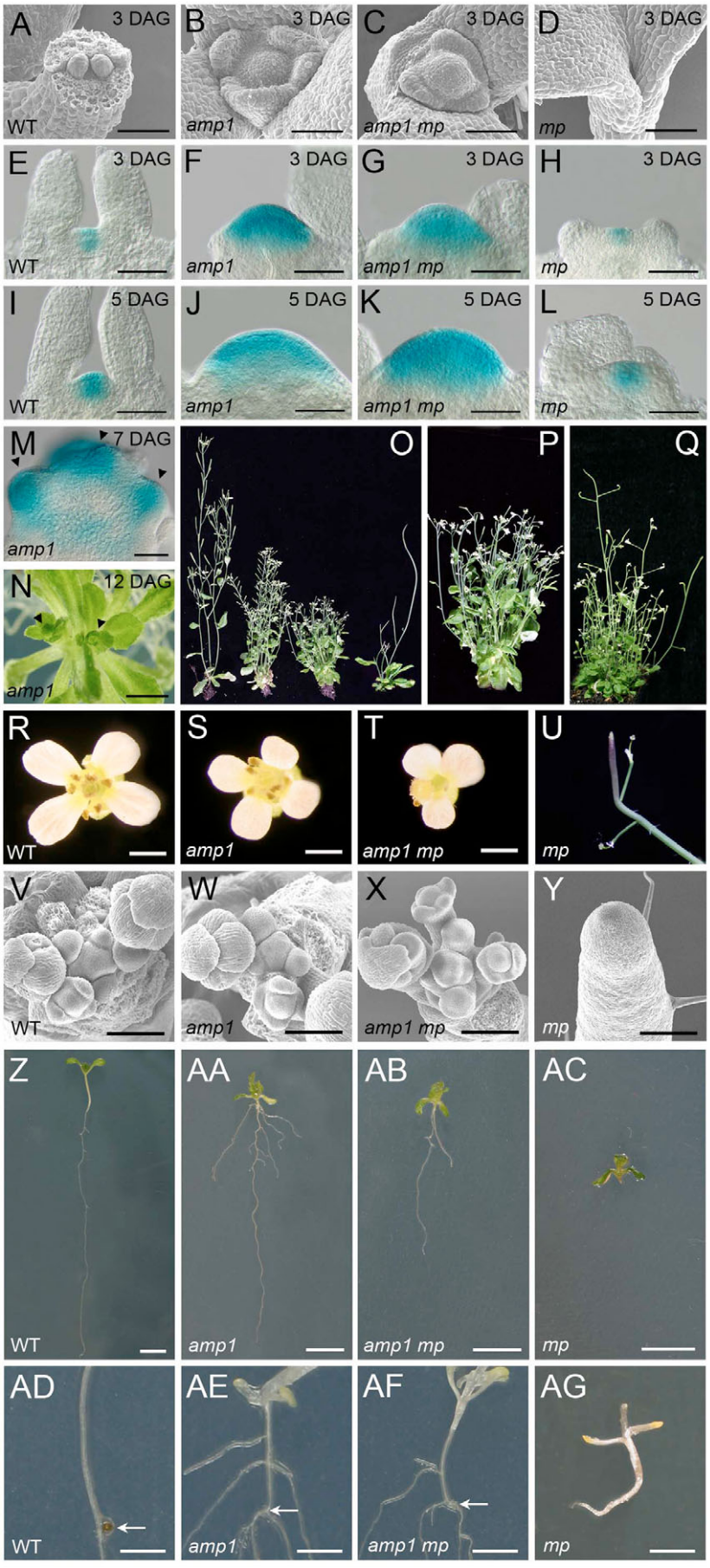

Fig. 2. Postembryonic interaction of *AMP1* and *MP* in *Arabidopsis*. (**A-D**) Scanning electron micrographs showing relative sizes of SAMs of indicated genotypes at 3 days after germination (DAG). (**E-L**) Cleared whole-mount preparations viewed with DIC optics. Expression of stem cell marker *pCLV3::GUS* in the SAM of the indicated genotypes at 3 DAG (E-H) and 5 DAG (I-L). Note that expression of *pCLV3::GUS* extends beyond the most-central domain in the *amp1* background. Numerical values of shoot meristem sizes are shown in Table 1A. (**M**) Discrete domains of *pCLV3::GUS* expression (arrowheads) in *amp1* are associated with the formation of multiple meristems. (**N**) Formation of multiple SAMs (arrowheads) on expanded apices in *amp1* mutants, which are clearly not associated with leaf axils. (**O**) Left to right: wild type, *amp1-9*, *amp1-9 mpG92* and *mpG92*. Note that *amp1 mp* double mutants are smaller than *amp1* mutants and have restored flower formation, which is defective in *mp* mutants. (**P**,**Q**) Gene dosage-specific phenotypes of *amp1 mp* double mutants. Note that the restoration of flower formation is far more complete in a double mutant comprising a strong *amp1* and a weak *mp* allele (*amp1-9 mpG92* in P) than in combination with a strong *mp* allele (*amp1-1 mpBS1354* in Q). (**R-U**) Flower phenotypes. Normal flower organs in *amp1* mutants (*amp1-9* in S), inflorescence with few, highly reduced flowers in *mpG92* mutants (U) and intermediate, fertile flowers in *amp1-9 mpG92* mutants (T). (**V-Y**) Scanning electron micrographs of inflorescences indicating that no size abnormalities were observed in *amp1* mutants. (**Z-AC**) Light-grown seedlings at 7 DAG. Note the advanced stage of the lateral root system in *amp1* mutants. (**AD-AG**) Dark-grown seedlings at 14 DAG. Adventitious roots formed in *amp1-9* and *amp1-9 mpG92* mutants (arrows point to hypocotyl-root junction). Scale bars: 100 μm in A-D,V-Y; 50 μm in E-M; 1 mm in R-T; 5 mm in Z-AC; 2 mm in N,AD-AG.

Table 1. Developmental features of wild-type and *amp1*, *mp* and *amp1 mp* mutant plants

A. Shoot meristem size

Measurement	Ler	*amp1-9*	*amp1-9 mpG92*	*mpG92*
Meristem size, 3 DAG				
Meristem area, μm^2	356±28 (32)	5548±551 (25)*	5220±593 (29)*	26±2 (49)*
Meristem width, μm	47±2 (32)	148±6 (25)*	152±7(29)*	14±3 (49)*
Meristem height, μm	11±1 (32)	54±4 (25)*	52±4 (29)*	3±1 (49)*
Meristem size, 5 DAG				
Meristem area, μm^2	589±77 (28)	9214±988 (18)*	8766±902 (23)*	211±23 (26)*
Meristem width, μm	62±3 (28)	196±7 (18)*	190±9 (23)*	40±3 (26)*
Meristem height, μm	14±1 (28)	70±6 (18)*	68±6 (23)*	8±2 (26)*

Values are mean±s.e.m., (*n*). Note that only (13/49) and (10/26) *mpG92* mutants produced shoot apical meristems (SAMs) at 3 days after germination (DAG) and 5 DAG, respectively. Mutants that failed to produce a SAM were included in the calculations and given a measurement value of zero since all mutants will form SAMs subsequently.
*Significantly (P<0.05) different from wild-type population, based on Student's *t*-test.

B. Frequency of adventitious root formation

Measurement	Ler	*amp1-9*	*amp1-9 mpG92*	*mpG92*
No. adv roots, 14 DAG, dark	0.2±0.1 (55)	3.4±0.2 (51)†	1.5±0.1 (46)†	0 (39)†
No. adv roots, 7 DAG, light	1.0±0.6 (21)	2.2±1.1 (26)†	1.2±0.7 (45)	0 (36)†

Values are mean number of adventitious roots from the hypocotyl±s.e.m., (*n*).
†Significantly (P<0.05) different from wild-type population, based on Student's *t*-test.

C. Early emergence of lateral roots is observed in *amp1* seedlings at 4 DAG

Genotype	*n*	Number of initiation sites
WT	39	0.1±0.05
amp1-9	37	3.0±0.21‡
amp1-9 mpG92	37	0.5±0.12‡
mpG92	40	0±0

Results shown are the average number of lateral roots (initiation sites) marked by *CycB1,1::GUS* expression in the primary root±s.e.m. at 4 DAG.
‡Significantly (P<0.05) different from wild-type (WT) population, based on Student's *t*-test.

D. Restoration of seedling pattern (rooted seedlings with hypocotyls at 10 DAG)

Genotype	No. of rooting individuals	Total (*n*)	Frequency of rooting (%)
amp1-1 mpBS1354	35	338	10.3
amp1-1 mpG12	8	195	4.1
amp1-1 mpG33	12	136	8.8
amp1-11 mpBS1354	57	147	38.8
amp1-10 mpG12	40	132	30.3
amp1-8 mpG92	64	197	32.5
amp1-9 mpG92	26	165	15.8

E. Floral organ numbers of *amp1 mp* double mutants

		Total		Sepals		Petals		Stamens		Carpels	
Genotype	*n*	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Ler	50	4	4.0	4	4.0	5-6	5.8±0.1	2	2.0	15-16	15.8±0.1
amp1-9	50	4	4.0	4	4.0	5-6	5.9±0.1	2	2.0	15-16	15.9±0.1
amp1-9 mpG92	40	0-4	2.4±0.1§	2-8	3.5±0.2§	1-4	2.7±0.2§	1-2	1.6±0.1§	7-15	10.2±0.2§
amp1-8	50	4	4.0	4	4.0	5-7	5.9±0.1	2	2.0	15-17	15.9±0.1
amp1-8 mpBS62	50	2-4	3.7±0.1§	2-4	3.6±0.1§	4-6	5.0±0.1§	1-2	1.9±0.1	11-16	14.2±0.2§

Values are range and mean number of floral organs produced±s.e.m.
§Significantly (P<0.05) different from wild-type population, based on Student's *t*-test.

F. Apical pattern phenotypes of *amp1 mp* double mutants

Genotype	*n*	Monocot %	Fused %	> 2 cots %	Other %
amp1-11 mpBS1354	88	23.9	14.8	61.3	
amp1-8 mpBS62	181	2.7	3.9	92.8	0.6
amp1-9 mpG92	125	20.4	4.7	64.6	10.3
mpBS1354	92	50.0	4.3	44.6	1.1
mpBS62	74	35.1	8.1	56.8	
mpG92	107	14.9	25.3	59.8	

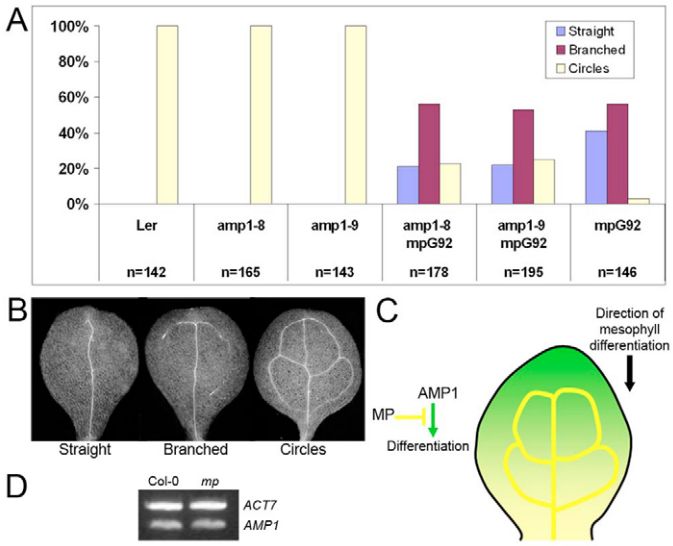

Fig. 3. Vascular systems in cotyledons of wild-type and mutant *Arabidopsis* seedlings. (**A**,**B**) Vascular phenotype categories: 'straight', referring to the formation of a single unbranched midvein; 'branched', a complete midvein plus some secondary venation; 'circles', complete midvein and at least one second-order vein has formed a complete loop. Frequencies of vascular phenotypic categories (shown in B) in various genotypes. Note the higher frequencies of more-complete vascular systems in *amp1 mp* double mutants as compared with *mp* mutants. One cotyledon per seedling was evaluated. (**C**) Scheme of *AMP1* interaction with *MP*. As illustrated for cell fate acquisition in the leaf primordium, but applicable also to other locations, *AMP1* functions as a universal negative regulator of meristematic activity (promoter of cell differentation). Locally interfering with *AMP1* function, *MP* maintains cells in a procambial state by preventing the acquisition of mesophyll characteristics (green) along lines of elevated auxin levels and *MP* expression (yellow). (**D**) RT-PCR of *AMP1* transcript abundance in wild-type and *mpG92* seedlings at 7 days after germination (DAG). *ACT7* was used as an internal control as described (Hardtke et al., 2004).

Genetic interaction between *AMP1* and *MP*

Our observations suggest a model in which auxin-derived positional information through *MP* carves out meristematic niches by locally overcoming a general differentiation-promoting activity involving *AMP1* (Fig. 3C). This model is consistent with the overall meristem-promoting activity of *MP* contrasted by the differentiation-promoting activity of *AMP1*, but clearly involves more than the superimposition of antagonistic phenotypes. First, *amp1* also suppresses *mp* where it does not confer an opposite phenotype. For example, *amp1* increases vascular complexity only in an *mp*, not in a wild-type, background and restores flower formation in *mp* mutant inflorescence meristems, whereas *amp1* inflorescence meristems are phenotypically normal. Conversely, a mutation associated with enlarged meristems, such as *clavata 2*, does not necessarily affect *mp* meristem phenotypes (see Fig. S2 in the supplementary material). Therefore, interaction of *AMP1* and *MP* is not simply a consequence of opposite controls of meristem sizes. Instead *MP* seems to overcome *AMP1* activity in meristematic regions and therefore becomes (partially) irrelevant in the absence of AMP1 activity (Fig. 3C).

We provide further evidence for similarities between AMP1 and mammalian NAALADases, including intracellular location and tunicamycin sensitivity (see Figs S3, S4 in the supplementary material). Because targets and pathways of mammalian NAALADases have turned out to be extremely difficult to unravel (Zhou et al., 2005), molecular details of the *AMP1* pathway and the nature of the interaction with *MP* might also not be immediately tractable. At this point, we can rule out three obvious possibilities for this interaction. First, as explained above, the interaction cannot be reduced to the superimposition of opposite controls. Second, *MP* does not seem to overcome *AMP1* activity by transcriptional downregulation of *AMP1*, because *AMP1* transcript levels are unaffected in *mp* seedlings (Fig. 3D). Third, MP and AMP1 are unlikely to interact physically, as they are localized to different cellular compartments (see Fig. S4B,C in the supplementary material). This finding is also reflected in the absence of semidominant suppressive effects, which are frequently associated with direct interaction, in *amp1 mp* double mutants. Both gene products are, however, co-expressed in many locations and their respective pathways could therefore interact in those cells (see Fig. S5 in the supplementary material).

In summary, mutant phenotypes and expression patterns suggest that *MP* locally interferes with *AMP1*-promoted cell differentiation to maintain meristematic niches.

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/134/14/2561/DC1

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